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PATENT- OG VAREMÆRKESTYRELSEN

Therapeutic use of a growth factor, NsG33

TECHNICAL FIELD

The present invention relates to the field of therapeutic use of proteins, genes and cells, in particular to the therapy based on the biological function of a secreted therapeutic protein, NsG33, in particular for the treatment of disorders of the nervous system and for the treatment immunological disorders. The invention also relates to bioactive NsG33 polypeptide fragments and the corresponding encoding DNA sequences.

BACKGROUND ART

Extracellular proteins play important roles in, among other things, the formation, differentiation and maintenance of multicellular organisms. The fate of many individual cells, e.g., growth including proliferation, migration, differentiation, or interaction with other cells, is typically governed by information received from other cells and/or the immediate environment. This information is often transmitted by secreted polypeptides (for instance, mitogenic factors, survival factors, cytotoxic factors, differentiation factors, neuropeptides, and hormones) which are, in turn, received and interpreted by diverse cell receptors or membrane-bound proteins. These secreted polypeptides or signaling molecules normally pass through the cellular secretory pathway to reach their site of action in the extracellular environment.

Disorders such as Parkinson's disease, Alzheimer's disease, Huntington's disease, multiple and amyotrophic lateral sclerosis, stroke, schizophrenia, epilepsy and peripheral neuropathy and associated pain affect millions of people. It is the loss of normal neuronal function, which produces the behavioral and physical deficits which are characteristic of each of the different neurological disorders. In addition to chronic and acute neurodegenerative disorders, the aging process, physical trauma to the nervous system, and metabolic disorders may result in the loss, dysfunction, or degeneration of neural cells accompanied by the associated behavioral and physical deficits. Many of these diseases are today incurable, highly debilitating, and traditional drug therapies often fail. There is thus a great medical need for new therapeutic proteins that are disease modifying and not only for symptomatic use.

Several secreted factors with expression in the nervous system or associated target areas have important therapeutic uses in various neurological indications associated with reduction or loss of neuronal functions. E.g. NGF is a candidate for treatment of Alzheimer's disease, Neublastin

(Artemin) a candidate for treatment of peripheral neuropathy, and GDNF is a candidate for treatment of Parkinson's Disease.

SUMMARY OF THE INVENTION

In a first aspect the invention relates to an isolated polypeptide for medical use, said polypeptide comprising an amino acid sequence selected from the group consisting of:
a) the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 8, 9, 10, 13, 14, 15, 19, 20, 21, 22, 23, and 24;
b) a sequence variant of the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 8, 9, 10, 13, 14, 15, 19, 20, 21, 22, 23, and 24, wherein the variant has at least 70% sequence identity to said SEQ ID No.; and
c) a biologically active fragment of at least 50 contiguous amino acids of any of a) through b).

Based on the finding that NsG33 is a secreted protein with growth factor characteristics, which is expressed at high levels and selectively in the nervous system and the eye, and especially in substantia nigra, the putamen and spinal cord, as well as in the mesencephalon of the developing human embryo, the present inventors contemplate use of NsG33 in the treatment of disorders of the central nervous system, in particular in the treatment of Parkinson's Disease, Huntington's disease, and disorders of the spinal cord, such as ALS. Based on the expression in the cerebellum, the dorsal root ganglion and the retina, NsG33 is also contemplated for use in the treatment of peripheral neuropathies and associated pain, as well as cerebellar disorders and retinopathies.

Other therapeutically relevant secreted growth factors are expressed in the nervous system or subregions thereof including but not limited to GDNF, NGF, Neurturin, BDNF, NT4/5, NT3, Neublastin (Artemin).

Based on sequence identity to a protein disclosed in WO 93/22437, NsG33 is contemplated for use in the treatment of immunological disorders.

The therapeutic effect of NsG33 may be mediated through an effect on growth, survival, regeneration, regain or improvement of function, and/or on differentiation of targeted cells.

In a further aspect the invention relates to an isolated nucleic acid molecule for medical use comprising a nucleic acid sequence encoding a polypeptide, or the complementary sequence of

the encoding sequence, said polypeptide comprising an amino acid sequence selected from the group consisting of:

a) the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 8, 9, 10, 13, 14, 15, 19, 20, 21, 22, 23, and 24;

b) a sequence variant of the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 8, 9, 10, 13, 14, 15, 19, 20, 21, 22, 23, and 24, wherein the variant has at least 70% sequence identity to said SEQ ID No.; and

c) a biologically active fragment of at least 50 contiguous amino acids of any of a) through b).

In a further aspect the invention relates to an isolated nucleic acid molecule for medical use, wherein the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of

a) the nucleotide sequence selected from the group consisting of SEQ ID No. 1, 2, 6, 7, 11, 12, 16, 17, and 18;

b) a nucleotide sequence having at least 50% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID No. 1, 2, 6, 7, 11, 12, 16, 17, and 18;

c) a nucleic acid sequence of at least 150 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID No. 1, 2, 6, 7, 11, 12, 16, 17, and 18;

c) the complement of a nucleic acid capable of hybridising with nucleic acid having the sequence selected from the group consisting of SEQ ID No. 1, 2, 6, 7, 11, 12, 16, 17, and 18 under conditions of high stringency; and

d) the nucleic acid sequence of the complement of any of the above.

In a further aspect the invention relates to an expression vector comprising a nucleic acid molecule of the invention.

In a still further aspect the invention relates to an isolated host cell comprising an expression vector according to the invention. In particular the invenction relates to host cells useful for cell based therapy, either naked cell base therapy or encapsulated cell therapy.

In a further aspect the invention relates to a packaging cell line capable of producing an infective virus particle, said virus particle comprising a Retroviridae derived genome comprising a 5' retroviral LTR, a tRNA binding site, a packaging signal, a promoter operably linked to a polynucleotide sequence encoding a polypeptide of the invention, an origin of second strand DNA synthesis, and a 3' retroviral LTR.

In a further aspect the invention relates to an implantable biocompatible cell device, the device comprising:

i) a semipermeable membrane permitting the diffusion of a protein of the invention; and

ii) a composition of cells according to the invention, or a composition of packaging cells according to the invention.

In a further aspect the invention relates to a pharmaceutical composition comprising

i) a polypeptide of the invention; or

ii) an isolated nucleic acid sequence of the invention; or

iii) an expression vector of the invention; or

iv) a composition of host cells according to the invention; or

v) a packaging cell line according to the invention; or

vi) an implantable biocompatible cell device according to the invention; and

a pharmaceutically acceptable carrier.

In a further aspect the invention relates to the use of

i) a polypeptide of the invention; or

ii) an isolated nucleic acid sequence of the invention; or

iii) an expression vector of the invention; or

iv) a composition of host cells according to the invention; or

v) a packaging cell line according to the invention; or

vi) an implantable biocompatible capsule according to the invention.

for the manufacture of a medicament.

In a further aspect the invention relates to a method of treatment of a pathological condition in a subject, comprising administering to a subject in need therof a therapeutically effective amount of:

i) a polypeptide of the invention; or

ii) an isolated nucleic acid sequence of the invention; or

iii) an expression vector of the invention; or

iv) a composition of host cells according to the invention; or

v) a packaging cell line according to the invention; or

vi) an implantable biocompatible capsule according to the invention.

In a further aspect the invention relates to the use of

i) a polypeptide of the invention; or

ii) an isolated nucleic acid sequence of the invention; or

iii) an expression vector of the invention; or

iv) a composition of host cells according to the invention; or

v) a packaging cell line according to the invention;

as a growth factor in mammalian cell culture.

In one aspect the invention relates to an antibody capable of binding to a polypeptide of the invention.

In a further aspect the invention relates to an immunoconjugate comprising the antibody of the invention and a conjugate selected from the group consisting of: a cytotoxic agent such as a chemotherapeutic agent, a toxin, or a radioactive isotope; a member of a specific binding pair, such as avidin or streptavidin or an antigen; an enzyme capable of producing a detectable product.

In a further aspect the invention relates to an isolated polypeptide selected from the group consisting of AA_{128}-AA_{293} of SEQ ID No 3, AA_{121}-AA_{293} of SEQ ID No 3, AA_{129}-AA_{294} of SEQ ID No 8, AA_{122}-AA_{294} of SEQ ID No 8, AA_{126}-AA_{291} of SEQ ID No 13, AA_{119}-AA_{291} of SEQ ID No 13, and variant of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 15 of the amino acid residues in the sequence are so changed. These isolated polypeptides constitute C-terminal peptides of NsG33. Preferably any changed amino acids are selected from those designated as unconserved, weakly conserved or strongly conserved in Figure 3.

In a further aspect the invention relates to specific truncated forms of NsG33. In one aspect these are selected from the group consisting of:

1) AA_{30}-AA_{288} of SEQ ID No 3, and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{25}-AA_{293} of SEQ ID No 3;

2) AA_{28}-AA_{286} of SEQ ID No 13 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{23}-AA_{291} of SEQ ID No 13;

3) AA_{31}-AA_{289} of SEQ ID No 8 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{26}-AA_{294} of SEQ ID No 8; and

4) variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 20 of the amino acid residues in the sequence are so changed.

These truncated forms of NsG33 constitute a bioactive core sequence from the first to the last conserved cysteine.

In a further aspect the invention relates to specific truncated forms of NsG33. In one aspect these are selected from the group consisting of:

1) AA_{171}-AA_{288} of SEQ ID No 3, and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{165}-AA_{288} of SEQ ID No 3;

2) AA_{169}-AA_{286} of SEQ ID No 13 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{164}-AA_{291} of SEQ ID No 13;

3) AA_{172}-AA_{289} of SEQ ID No 8 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, i.e. up to AA_{167}-AA_{294} of SEQ ID No 8;

4) variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed.

These truncated forms constitute a bioactive core sequence of the C-terminal NsG33 peptides from the first to the last conserved cysteine in the C-terminal peptides.

In a further aspect the invention relates to specific truncated forms of NsG33. In one aspect these are selected from the group consisting of:

1) AA_{30}-AA_{118} of SEQ ID No 3, and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{25}-AA_{123} of SEQ ID No 3;

2) AA_{28}-AA_{116} of SEQ ID No 13 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{23}-AA_{121} of SEQ ID No 13;

3) AA_{31}-AA_{119} of SEQ ID No 8 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{26}-AA_{124} of SEQ ID No 8; and

4) variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed.

These truncated forms constitute a bioactive core sequence of the N-terminal NsG33 peptides from the first to the last conserved cysteine in the N-terminal peptides.

The invention also relates to nucleic acids coding for said C-terminal, N-terminal and truncated NsG33 as well as vectors comprising the nucleic acids coding for these, cells capable of producing these, and methods of preparing said C-terminal, N-terminal and truncated NsG33.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1a: SignalP NN plot of human NsG33. Figure 1b: SignalP HMM plot of human NsG33.

Figure 2: Output from the ProtFun2.1 protein function prediction server on full length human NsG33 (SEQ ID No 3), human N-terminal peptide (SEQ ID No 19) and human C-terminal peptide (SEQ ID No 5).

Figure 3: Clustal W (1.82) multiple sequence alignment of human, mouse and rat NsG33. The signal sequences are shown in bold. A putative furin cleavage site has been underlined.

* indicates positions which have a single, fully conserved residue.

: indicates that one of the following 'strong' groups is fully conserved:
-STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW.

. indicates that one of the following 'weaker' groups is fully conserved:
-CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY.

Figure 4: Real Time PCR on NsG33. For details see Example 5.
Figure 4, upper panel shows the relative expression of NsG33 (relative to tissue with the lowest expression) assuming same amounts of cDNA were synthesized from equal amounts of total RNA used for the cDNA step.
Figure 4, lower panel shows the relative expression of NsG33 normalised to $ß_2$-microglobulin (relative to tissue with the lowest normalized expression). Results should be interpreted with caution as $ß_2$-microglobulin expression levels vary between some tissues.

Figure 5: Align0 alignment of full length human NsG33 polypeptide against full-length human polypeptide (Innog.) from WO 93/22437 (Innogenetics SA). Scoring matrix BLOSUM50, gap penalties: -12/-2. The ten conserved cysteines are shown in bold with asterisks above or below the aligned sequences.

Figure 6: Human NsG33 cDNA and encoded prepro-NsG33

Figure 7: Partial mouse NsG33 cDNA and encoded partial pre-pro-NsG33

Figure 8: Rat NsG33 cDNA and encoded pre-pro-NsG33.

Definitions:

NsG33, as used herein, refers to polypeptides having the amino acid sequences of substantially purified NsG33 obtained from any species, particularly mammalian, including chimpanzee, bovine, ovine, porcine, murine, equine, and preferably human, from any source whether natural, synthetic, semi-synthetic, or recombinant. The term also refers to biologically active fragments of NsG33 obtained from any of these species, as well as to biologically active sequence variants of these and to proteins subject to posttranslational modifications.

Growth factor characteristics as used herein define sequence-related features similar to those of classical growth factors, which are secreted proteins acting on a target cell through a receptor to cause one or more of the following responses in the target cell: growth including proliferation, differentiation, survival, regeneration, migration, regain of function, improvement of function.

An "allele" or "allelic sequence", as used herein, is an alternative form of the gene encoding NsG33. Alleles may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

A "deletion", as used herein, refers to a change in the amino acid or nucleotide sequence and results in the absence of one or more amino acid residues or nucleotides.

An "insertion" or "addition", as used herein, refers to a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, as compared to the naturally occurring molecule.

The terms "specific binding" or "specifically binding", as used herein, refers to the high affinity interaction between a protein or peptide and a binding molecule such as an antibody and a receptor or fragments thereof. The interaction is dependent upon the presence of a particular structure (i.e., the antigenic determinant or epitope) of the protein recognized by the binding molecule. For example, if an antibody is specific for epitope "A", the presence of a protein containing epitope A (or free, unlabeled A) in a reaction containing labeled "A" and the antibody will reduce the amount of labeled A bound to the antibody.

The term "substantially purified", as used herein, refers to nucleic or amino acid sequences that

are removed from their natural environment, isolated or separated, and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated.

A "substitution", as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Sequence identity":

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264-2268, modified as in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5877. Such an algorithm is incorporated into the BLASTN and BLASTP programs of Altschul, et al. (1990) J. Mol. Biol. 215:403-410.

In order to characterize the identity, subject sequences are aligned so that the highest order homology (match) is obtained. Based on these general principles the "percent identity" of two amino acid sequences may be determined using the BLASTP algorithm [*Tatiana A. Tatusova, Thomas L. Madden*: Blast 2 sequences - a new tool for comparing protein and nucleotide sequences; FEMS Microbiol. Lett. 1999 174 247-250], which is available from the National Center for Biotechnology Information (NCBI) web site (http://www.ncbi.nlm.nih.gov), and using the default settings suggested here (i.e. Matrix = Blosum62; Open gap = 11; Extension gap = 1; Penalties gap x_dropoff = 50; Expect = 10; Word size = 3; Filter on). The BLAST algorithm performs a two-step operation by first aligning two sequences based on the settings and then determining the % sequence identity in a range of overlap between two aligned sequences. In addition to % sequence identity, BLASTP also determines the % sequence similarity based on the settings.

In order to characterize the identity, subject sequences are aligned so that the highest order homology (match) is obtained. Based on these general principles, the "percent identity" of two nucleic acid sequences may be determined using the BLASTN algorithm [*Tatiana A. Tatusova, Thomas L. Madden*: Blast 2 sequences - a new tool for comparing protein and nucleotide sequences; FEMS Microbiol. Lett. 1999 174 247-250], which is available from the National Center for Biotechnology Information (NCBI) web site (http://www.ncbi.nlm.nih.gov), and using the default settings suggested here (i.e. Reward for a match = 1; Penalty for a match = -2; Strand option = both strands; Open gap = 5; Extension gap = 2; Penalties gap x_dropoff = 50;

Expect = 10; Word size = 11; Filter on). The BLASTN algorithm determines the % sequence identity in a range of overlap between two aligned nucleotide sequences.

Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the FASTA sequence alignment software package (Pearson WR, Methods Mol Biol, 2000, 132:185-219). Align calculates sequence identities based on a global alignment. Align0 does not penalise to gaps in the end of the sequences. When utilizing the ALIGN og Align0 program for comparing amino acid sequences, a BLOSUM50 substitution matrix with gap opening/extension penalties of –12/-2 is preferably used.

DETAILED DESCRIPTION

The present invention relates to the medical use of polypeptides and polynucleotides being identified as NsG33. The NsG33 protein has been identified in human beings (SEQ ID No. 3), mouse (SEQ ID No. 8), and rat (SEQ ID No. 13).

Human NsG33 exists as a 293 amino acid precursor, which can be processed to give rise to several biologically active peptides. NsG33 is expressed at high levels in the nervous system and the eye, and in particular subregions of the brain (Figure 4A and B). The longest mouse (SEQ ID No 8) and rat (SEQ ID No 13) NsG33 polypeptides consist of 294 and 291 amino acids, respectively and the % identities with the human protein are 80.3 and 80.2, respectively (See Table 1 and 2 in Example 2). It should be noted that the predicted full length mouse and rat polypeptide sequences are as yet unverified, and that at least the mouse polypeptide sequences is partial in the N-terminal.

Human NsG33 contains an N-terminal signal peptide sequence of 23 amino acids, which is cleaved at the sequence motif ARA-GY. This signal peptide cleavage site is predicted by the SignalP method (Nielsen *et al.*,1997) and the output graph shown in FIG.1. However, one of skilled in the art will recognize that the actual cleavage site may be different than predicted by the computer program. For example the signal peptide prediction in rat NsG33 results in predictions with approximately equal probabilities at positon 16 and 21. A signal peptide cleavage site is found at a similar location in the mouse NsG33 (pos. 24) and rat NsG33 (pos. 16 or 21).

General-type proprotein cleavage is predicted in human NsG33 by the ProP method (Prediction of proprotein convertase cleavage sites. *Peter Duckert, Søren Brunak and Nikolaj Blom.* Protein Engineering, Design and Selection: 17: 107-112, 2004) at pos. 127 with a score of 0.831, sequence motif 'WGPRERR-AL'. Similar, cleavage sites are predicted in homologous positions in mouse NsG33 (at pos. 128) with a score of 0.831, sequence motif 'WGPRERR-AL' and in rat NsG33 (at pos. 125) with a score of 0.831 and the sequence motif 'WGPRERR-AL'. A possible furin propeptide cleavage site is also found at position 121 in human NsG33 at sequence motif 'GGRCVR-WG' and at corresponding positions in rat and mouse NsG33.

Polypeptide processing after cleavage of the signal peptide results in the formation of a C-terminal peptide and an N-terminal peptide.

NsG33 belongs to the category of proteins acting as growth factors. This notion is supported by predictions by the ProtFun protein function prediction server (Jensen *et al.*, 2002 & 2003), which provides odds above 1.0 this type of category as shown in FIG. 2.

The ProtFun method predicts protein function based on sequence-derived features as opposed to sequence similarity. Features which are important for discriminating between the 'growth factor' classes versus all other classes are: protein sorting potential, protein targeting potential, signal peptide potential, low complexity regions, secondary protein structure, number of negative residues and number of atoms (Jensen *et al.*, 2003). In general, an odds score of 1 indicates a prediction which may have taken place by chance. Odds above 1 indicate that there is an increased probability that the protein does belong to the predicted gene ontology class. The higher the odds score, the higher the chance that the prediction is correct.

Results of the QRT-PCR are shown in Figures 4 A and B.

Tissues with high expression included:
Putamen, Substantia Nigra and Spinal Cord.
Tissues with intermediate expression included:
Whole brain, Cerebellum, Retina* and Dorsal Root Ganglion*
Tissues with low expression included:
Heart, kidney, Lung, Prostate, Salivary gland, skeletal muscle, testis, stomach, pancreas, Fetal Brain*.
Tissues with very low or no expression included:
Fetal Liver, Placenta, thymus, trachea, spleen, uterus, colon, small intestine.

When analysing results after normalisation to β_2-microglobulin expression essentially same results were seen except for the tissues marked with a *

Unlike structural proteins, growth factors are involved in cell signalling and in various functions such as growth, proliferation, differentiation, survival, regeneration, migration, regain of function and improvement of function. Therefore, growth factors can be administred and be used to exert a therapeutic effect. Based on the tissue specific expression, and the fact that NsG33 is predicted to be a secreted growth factor, NsG33 is contemplated for use in treating disorders of the nervous system in general (based on the nervous sytem specific expression), in particular Parkinson's disease (based on the expression in substantia nigra), Huntingtons disease (based on expression in putamen and substantia nigra), cerebellar disorders (based on expression in cerebellum), Spinal Cord injury (based on expression in the spinal cord), ALS (based on expression in the spinal cord), peripheral neuropathies (based on expression in dorsal root ganglion), and retinopathies (based on expression in retina). The function for the various indications can be verified in in vitro and in vivo assays as described in the examples.

Likewise, expression of therapeutically relevant secreted growth factors including GDNF, NGF, and Neublastin (Artemin) is found in target areas of the neurological disorder they may be used to treat.

The therapeutic effect of NsG33 may be mediated through an effect on growth including proliferation, regeneration, regain of function, improvement of function, survival, migration, and/or differentiation of targeted cells.

NsG33 is structurally related to a protein described in WO 93/22437 (Innogenetics SA), which is identified in a BLASTP search. The full length human protein is shown in Figure 2 of WO 93/22437. NsG33 shares 42 % identity (Align0 with default settings) with the Innogenetics protein including 10 conserved cysteine residues. An N-terminal signal peptide of 45 residues is predicted in NsG34. Recombinant expression data from WO 93/22437 indicate that the Innogenetics protein is not subject to pro-peptide processing under the conditions used in that publication, but is secreted as a protein of 268 amino acids.

A full length alignment of human NsG33 to the human Innogenetics protein is shown in Figure 5. The 10 conserved cysteines are shown in bold and are marked with asterisks. The two proteins together form a protein family based on the conserved cysteine residues and the stretches of

high conservation which are evident from Figure 5. None of the two proteins show any significant sequence homology to any other known human proteins. Although the two proteins are members of the same small protein family, the two proteins are structurally distinct.

Due to the high conservation of the cysteines, it is expected that these residues play an important role in the secondary and tertiary structure of the bioactive protein. One or more of the cysteines may participate in the formation of intra- and/or intermolecular cystin-bridges.

The Innogenetics protein is inter alia functional in activation of T-cells and B-cells and as an inducer of immunosuppressive cells. Based on the homology to the Innogenetics protein, NsG33 is predicted to similarly have functions involved in immunology.

I NSG33 POLYPEPTIDES

In addition to full-length NsG33, substantially full-length NsG33, to pro-NsG33, to C-terminal peptides, to N-terminal peptides and to truncated forms of NsG33, the present invention provides for biologically active variants of the polypeptides. An NsG33 polypeptide or fragment is biologically active if it exhibits a biological activity of naturally occurring NsG33. It is to be understood that the invention relates to substantially purified NsG33 as herein defined.

One biological activity is the ability to compete with naturally occurring NsG33 in a receptor-binding assay.

Another biological activity is the ability to bind to an antibody, which is directed at an epitope, which is present on naturally occurring NsG33.

Biologically active variants may also be defined with reference to one or more of the other in vitro and/or in vivo biological assays described in the examples.

A preferred biological activity is the ability to elicit substantially the same response as in the PC12 assay described in the Examples. In this assay PC12 cells are transduced with full length human NsG33 coding sequence (Figure 6). By substantially the same response in the PC12 assay is intended that the number of neurite bearing cells is at least 10% of the number obtained in Example 6 (transduction with full length human NsG33), more preferably at least 20%, more preferably at least 30%, more preferably at least 40%, more preferably at least 50%, more preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more

preferably at least 80%, more preferably at least 85%, more preferably at least 90%. The PC12 assay may also be used to document the percentage improvement in survival over a control treatment. Substantially the same response in this context means an activity resulting in at least 10% of the improvement obtained in Example 6, more preferably at least 20%, more preferably at least 30%, more preferably at least 40%, more preferably at least 50%, more preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 90%. The biological activity of a fragment or variant of NsG33 may also be higher than that of the naturally occurring NsG33.

Specific fragments of NsG33 include polypeptides selected from the group consisting of AA_{128}-AA_{293} of SEQ ID No 3, AA_{121}-AA_{293} of SEQ ID No 3, AA_{129}-AA_{294} of SEQ ID No 8, AA_{122}-AA_{294} of SEQ ID No 8, AA_{126}-AA_{291} of SEQ ID No 13, AA_{119}-AA_{291} of SEQ ID No 13, and sequence variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 15 of the amino acid residues in the sequence are so changed. These isolated polypeptides constitute C-terminal peptides of NsG33. Preferably any changed amino acids are selected from those designated as unconserved, weakly conserved or strongly conserved in Figure 3. ProtFun 2.1 predicts with high odds (8.0) that C-terminal peptides belong to the gene ontology class growth factor (Figure 2).

Further specific polypeptides are selected from the group consisting of SEQ ID No 19, 20, 21, 22, 23, and 24, and sequence variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 15 of the amino acid residues in the sequence are so changed. These isolated polypeptides constitute N-terminal peptides of NsG33. Preferably any changed amino acids are selected from those designated as unconserved, weakly conserved or strongly conserved in Figure 3. In a preferred embodiment, less than 10 amino acids have been changed, more preferably less than 5 amino acids, more preferably 1 or 2 amino acids, more preferably no amino acids have been changed. ProtFun 2.1 predicts with high odds (8.1) that N-terminal peptides belong to the gene ontology class growth factor (Figure 2).

Specific truncated forms of NsG33 in one aspect, are selected from the group consisting of:
1) AA_{30}-AA_{288} of SEQ ID No 3, and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{25}-AA_{293} of SEQ ID No 3;
2) AA_{28}-AA_{286} of SEQ ID No 13 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{23}-AA_{291} of SEQ ID No 13;

3) AA_{31}-AA_{289} of SEQ ID No 8 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{26}-AA_{294} of SEQ ID No 8; and

4) sequence variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 20 of the amino acid residues in the sequence are so changed.

These truncated forms of NsG33 constitute a core sequence from the first to the last conserved cysteine. In a preferred embodiment, less than 15 amino acids have been changed, more preferably less than 10 amino acids, more preferably less than 5 amino acids, such as 1 or 2 amino acids, more preferably no amino acids have been changed.

Specific truncated forms of NsG33 in one aspect, are selected from the group consisting of:

1) AA_{171}-AA_{288} of SEQ ID No 3, and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{165}-AA_{288} of SEQ ID No 3;

2) AA_{169}-AA_{286} of SEQ ID No 13 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{164}-AA_{291} of SEQ ID No 13;

3) AA_{172}-AA_{289} of SEQ ID No 8 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, i.e. up to AA_{167}-AA_{294} of SEQ ID No 8;

4) variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed.

These truncated forms constitute a bioactive core sequence from the first to the last conserved cysteine in C-terminal peptides. In a preferred embodiment, less than 10 amino acids have been changed, more preferably less than 5 amino acids, more preferably 1 or 2 amino acids, more preferably no amino acids have been changed.

Specific truncated forms of NsG33 in one aspect are selected from the group consisting of:

1) AA_{30}-AA_{118} of SEQ ID No 3, and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{25}-AA_{123} of SEQ ID No 3;

2) AA_{28}-AA_{116} of SEQ ID No 13 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{23}-AA_{121} of SEQ ID No 13;

3) AA_{31}-AA_{119} of SEQ ID No 8 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{26}-AA_{124} of SEQ ID No 8; and

4) variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed.

These truncated forms constitute core sequences from the first to the fourth conserved cysteine in N-terminal NsG33 peptides. In a preferred embodiment, less than 10 amino acids have been changed, more preferably less than 5 amino acids, more preferably 1 or 2 amino acids, more preferably no amino acids have been changed.

Variants can differ from naturally occurring NsG33 in amino acid sequence or in ways that do not involve sequence, or in both ways. Variants in amino acid sequence ("sequence variants") are produced when one or more amino acids in naturally occurring NsG33 is substituted with a different natural amino acid, an amino acid derivative or non-native amino acid. Particularly preferred variants include naturally occurring NsG33, or biologically active fragments of naturally occurring NsG33, whose sequences differ from the wild type sequence by one or more conservative and/or semi-conservative amino acid substitutions, which typically have minimal influence on the secondary and tertiary structure and hydrophobic nature of the protein or peptide. Variants may also have sequences, which differ by one or more non-conservative amino acid substitutions, deletions or insertions, which do not abolish the NsG33 biological activity. The Clustal W alignment in Figure 3 can be used to predict which amino acid residues can be substituted without substantially affecting the biological acitivity of the protein.

Substutions within the following group (Clustal W, 'strong' conservation group) are to be regarded as conservative substitutions within the meaning of the present invention

-STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW.

Substutions within the following group (Clustal W, 'weak' conservation group) are to be regarded as semi-conservative substitutions within the meaning of the present invention

-CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY.

Other variants within the invention are those with modifications which increase peptide stability. Such variants may contain, for example, one or more nonpeptide bonds (which replace the peptide bonds) in the peptide sequence. Also included are: variants that include residues other than naturally occurring L-amino acids, such as D-amino acids or non-naturally occurring or synthetic amino acids such as beta or gamma amino acids and cyclic variants. Incorporation of D-instead of L-amino acids into the polypeptide may increase its resistance to proteases. See, e. g., U. S. Patent 5,219,990. Splice variants are specifically included in the invention.

When the result of a given substitution cannot be predicted with certainty, the derivatives may be readily assayed according to the methods disclosed herein to determine the presence or absence of biological activity.

Variants within the scope of the invention in one embodiment include proteins and peptides with amino acid sequences having at least 60 percent identity with human, murine or rat NsG33 (SEQ ID NO: 5, 10, and 15). More preferably the sequence identity is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %.

Variants within the scope of the invention in one embodiment include proteins and peptides with amino acid sequences having at least 60 percent identity with a polypeptide having the sequence of SEQ ID NO: 4, 19, or 14. More preferably the sequence identity is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %.

Variants within the scope of the invention in one embodiment include proteins and peptides with amino acid sequences having at least 60 percent identity with a polypeptide having the sequence of SEQ ID NO: 3, 8, or 13. More preferably the sequence identity is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %.

Variants within the scope of the invention in one embodiment include proteins and peptides with amino acid sequences having at least 60 percent identity with a polypeptide having the sequence of SEQ ID NO: 19, 20, or 21. More preferably the sequence identity is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %.

Variants within the scope of the invention in one embodiment include proteins and peptides with amino acid sequences having at least 60 percent identity with a polypeptide having the sequence of SEQ ID NO: 22, 23, or 24. More preferably the sequence identity is at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more

preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98 %.

In a preferred embodiment the sequence identity of the variant NsG33 is determined with reference to a human NsG33 polypeptide (SEQ ID No 3, 4, 5, 19 or 22).

For the purposes of determining homology the minimum length of comparison sequences will generally be at least 8 amino acid residues, usually at least 12 amino acid residues. For the purposes of the present invention, the percent sequence identity is preferably calculated in a range of overlap of at least 25 amino acids, more preferably at least 30 amino acids, more preferably at least 35, more preferably at least 40, more preferably at least 45, more preferably at least 50, more preferably at least 55, more preferably at least 60, such as at least 70, for example at least 80, such as at least 90, for example at least 100, such as at least 110, for example at least 120, such as at least 130, for example at least 150, the range being determined by BLASTP under default settings.

In one embodiment the percent sequence identity is calculated using global alignment (GAP or Align), so that the variant and SEQ ID sequences are aligned, the total number of identical amino acid residues calculated and divided by the length of the SEQ ID NO.

In one embodiment, a variant NsG33 comprises a naturally occurring allelic variant of the sequence selected from the group consisting of SEQ ID No 3, 4, 5, 8, 9, 10, 13, 14, and 15. Said allelic variant sequence may be an amino acid sequence that is the translation of a nucleic acid sequence differing by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID No 1, 2, 6, 7, 11, 12, 16, 17, and 18.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 3, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 4, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 5, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 8, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 9, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 10, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 13, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 14, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 15, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 19, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 20, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 21, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 22, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 23, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, the variants include proteins comprising an amino acid sequence having at least 60% sequence identity to SEQ ID NO 24, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98%.

In one embodiment, a variant NsG33 at corresponding positions comprises the residues marked in Figure 3 as fully conserved (*), more preferably a variant NsG33 also comprises at corresponding positions the residues marked in Figure 3 as strongly conserved (: strongly conserved groups include: STA, NEQK, NHQK, NEDQ, QHRK, MILV, MILF, HY FYW), more preferably a variant NsG33 also comprises at corresponding positions the residues marked in Figure 3 as less conserved (. less conserved groups include: CSA, ATV, SAG, STNK, STPA,

SGND, SNDEQK, NDEQHK, NEQHK, NEQHRK, VLIM, HFY). In particular, it is contemplated that the conserved cysteines (Figure 5) must be located at corresponding positions in a variant NsG33.

Non-sequence modifications may include, for example, in vivo or in vitro chemical derivatisation of portions of naturally occurring NsG33, as well as acetylation, methylation, phosphorylation, carboxylation, PEG-ylation, or glycosylation. Just as it is possible to replace substituents of the protein, it is also possible to substitute functional groups, which are bound to the protein with groups characterized by similar features. Such modifications do not alter primary sequence. These will initially be conservative, i.e., the replacement group will have approximately the same size, shape, hydrophobicity and charge as the original group.

Many amino acids, including the terminal amino acids, may be modified in a given polypeptide, either by natural processes such as glycosylation and other post-translational modifications, or by chemical modification techniques which are well known in the art. Among the known modifications which may be present in polypeptides of the present invention are, to name an illustrative few, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a polynucleotide or polynucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

Such modifications are well known to those of skill and have been described in great detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, for instance, are described in most basic texts, such as, for instance, I. E. Creighton, Proteins-Structure and Molecular Properties, 2nd Ed., W. H. Freeman and Company, New York, 1993. Many detailed reviews are available on this subject, such as, for example, those provided by Wold, F., in Posttranslational Covalent Modification of Proteins, B. C. Johnson, Ed., Academic Press, New York, pp 1-12, 1983; Seifter et al., Meth. Enzymol. 182: 626-646, 1990 and Rattan et al., Protein Synthesis: Posttranslational Modifications and Aging, Ann. N.Y. Acad. Sci. 663: 48-62, 1992.

In addition, the protein may comprise a protein tag to allow subsequent purification and optionally removal of the tag using an endopeptidase. The tag may also comprise a protease cleavage site to facilitate subsequent removal of the tag. Non-limiting examples of affinity tags include a polyhis tag, a GST tag, a HA tag, a Flag tag, a C-myc tag, a HSV tag, a V5 tag, a maltose binding protein tag, a cellulose binding domain tag. Preferably for production and purification, the tag is a polyhistag. Preferably, the tag is in the C-terminal portion of the protein.

The native signal sequence of NsG33 may also be replaced in order to increase secretion of the protein in recombinant production in other mammalian cell types.

It will be appreciated, as is well known and as noted above, that polypeptides are not always entirely linear. For instance, polypeptides may be branched as a result of ubiquitination, and they may be circular, with or without branching, generally as a result of posttranslational events, including natural processing events and events brought about by human manipulation which do not occur naturally. Circular, branched and branched circular polypeptides may be synthesized by non-translational natural processes and by entirely synthetic methods, as well and are all within the scope of the present invention.

Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. In fact, blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, is common in naturally occurring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention, as well. For instance, the amino terminal residue of polypeptides made in E. coli, prior to proteolytic processing, almost invariably will be N-formylmethionine.

The modifications that occur in a polypeptide often will be a function of how it is made. For polypeptides made by expressing a cloned gene in a host, for instance, the nature and extent of the modifications in large part will be determined by the host cell's posttranslational modification capacity and the modification signals present in the polypeptide amino acid sequence. For instance, glycosylation often does not occur in bacterial hosts such as E. coli. Accordingly, when glycosylation is desired, a polypeptide should be expressed in a glycosylating host, generally a eukaryotic cell. Insect cells often carry out the same posttranslational glycosylations as mammalian cells and, for this reason, insect cell expression systems have been developed to efficiently express mammalian proteins having native patterns of glycosylation, inter alia. Similar considerations apply to other modifications.

It will be appreciated that the same type of modification may be present to the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications.

In general, as used herein, the term polypeptide encompasses all such modifications, particularly those that are present in polypeptides synthesized by expressing a polynucleotide in a host cell.

Also included within the invention are agents, which specifically bind to a protein of the invention, or a fragment of such a protein. These agents include Ig fusion proteins and antibodies (including single chain, double chain, F_{ab} fragments, and others, whether native, humanized, primatized, or chimeric). Additional descriptions of these categories of agents are in WO 95/16709, the disclosure of which is herein incorporated by reference.

Antibodies refer to intact molecules as well as fragments thereof, such as F_{ab}, $F_{(ab')}$, and F_v, which are capable of binding the epitopic determinant. Antibodies that bind NsG33 polypeptides can be prepared using intact polypeptides or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal can be derived from the translation of RNA or synthesized chemically and can be conjugated to a carrier protein, if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin and thyroglobulin, keyhole limpet hemocyanin. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

Humanised antibodies, as used herein, refer to antibody molecules in which amino acids have been replaced in the non-antigen binding regions in order to more closely resemble a human antibody, while still retaining the original binding ability. Humanised antibodies may be used therapeutically to treat conditions, where it is desirable to limit or block the action of NsG33.

Also included within the scope of the present invention are immunoconjugates of antibodies and conjugates selected from the group consisting of: a cytotoxic agent such as a chemotherapeutic agent, a toxin, or a radioactive isotope; a member of a specific binding pair, such as avidin, or streptavidin, or an antigen; an enzyme capable of producing a detectable product. These immunoconjugates can be used to target the conjugates to cells expressing a NsG33 receptor.

Specific antibodies to any NsG33 are also useful in immunoassays to quantify the substance for which a given antibody has specificity. Specific antibodies to an NsG33 may also be bound to solid supports, such as beads or dishes, and used to remove the ligand from a solution, either for use in purifying the protein or in clearing it from the solution. Each of these techniques is routine to those of skill in the immunological arts.

Also with the scope of the present invention are NsG33 fusion proteins. An NsG33 fusion protein can be used to allow imaging of tissues which express a receptor for NsG33, or in the immunohistological or preparative methods described above for antibodies to an NsG33. Fusion proteins encompassing an NsG33 can be used to specifically target medical therapies against cells, which express an NsG33 receptor.

II NSG33 NUCLEOTIDE SEQUENCES

The invention provides medical use of genomic DNA and cDNA coding for NsG33, including for example the human genomic nucleotide sequence (SEQ ID No. 1), the mouse and rat genomic sequences (SEQ ID No. 6 and 11), the nucleotide sequence of human, mouse and rat NsG33 cDNA (SEQ ID NO 2, 7, and 12) and the sequences coding for N-terminal NsG33 fragments of human, mouse, and rat origin (SEQ ID NO 16, SEQ ID No. 17, and SEQ ID No. 18).

Variants of these sequences are also included within the scope of the present invention.

The invention relates to an isolated nucleic acid molecule for medical use comprising a nucleic acid sequence encoding a polypeptide or its complementary sequence, said polypeptide comprising an amino acid sequence selected from the group consisting of:

a) the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 8, 9, 10, 13, 14, 15, 19, 20, 21, 22, 23, and 24;

b) a sequence variant of the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 8, 9, 10, 13, 14, 15, 19, 20, 21, 22, 23, and 24, wherein the variant has at least 70% sequence identity to said SEQ ID No.; and

c) a biologically active fragment of at least 50 contiguous amino acids of any of a) through b).

The nucleic acid molecule may comprise the nucleotide sequence of a naturally occurring allelic nucleic acid variant.

The nucleic acid molecule of the invention may encode a variant polypeptide, wherein the variant polypeptide has the polypeptide sequence of a naturally occurring polypeptide variant.

In one embodiment the nucleic acid molecule differs by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID No. 1, 2, 6, 7, 11, 12, 16, 17, and 18.

Preferably the encoded polypeptide has at least 60% sequence identity to a sequence selected from the group consisting of SEQ ID No. 5, 10, and 15 preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at leat 98% sequence identiy, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.s.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to a sequence selected from the group consisting of SEQ ID No. 3 and 4, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at leat 98% sequence identiy, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.s. Said sequences constitute human NsG33.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to a sequence selected from the group consisting of SEQ ID No. 19 and 22, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at leat 98% sequence identiy, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No.s. Said sequences constitute human NsG33.

In a preferred embodiment the encoded polypeptide has at least 60% sequence identity to the sequence of SEQ ID No. 5, preferably at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably, 75% sequence identity, more preferably at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90%

sequence identity, more preferably at least 95% sequence identity, more preferably at leat 98% sequence identiy, more preferably wherein the polypeptide has a sequence selected from the group consisting of said SEQ ID No. Said sequence constitutes human N-terminal NsG33 polypeptide.

In one aspect the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of

a) the nucleotide sequence selected from the group consisting of SEQ ID No. 1, 2, 6, 7, 11, 12, 16, 17, and 18;

b) a nucleotide sequence having at least 70% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID No. 1, 2, 6, 7, 11, 12, 16, 17, and 18;

c) a nucleic acid sequence of at least 150 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID No. 1, 2, 6, 7, 11, 12, 16, 17, and 18;

c) the complement of a nucleic acid capable of hybridising with nucleic acid having the sequence selected from the group consisting of SEQ ID No. 1, 2, 6, 7, 11, 12, 16, 17, and 18 under conditions of high stringency; and

d) the nucleic acid sequence of the complement of any of the above.

SEQ ID No 16, 17 and 18 represent the sequences coding for C-terminal NsG33 polypeptides from human, mouse and rat. For recombinant expression in a eukaryotic expression system, these are preferably ligated to appropriate signal sequence coding sequences to ensure that the NsG33 polypeptide is secreted from the cells.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO: 2, 7, 12, 16, 17, and 18.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO: 16, 17, and 18.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO: 2 and 16.

In one embodiment, the isolated polynucleotide of the invention has at least 60, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to the polynucleotide sequence presented as SEQ ID NO: 1.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence presented as SEQ ID NO: 2.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence presented as SEQ ID NO: 16.

In one embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence presented as SEQ ID NO: 6.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence presented as SEQ ID NO: 7.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence presented as SEQ ID NO: 17.

In one embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence presented as SEQ ID NO: 11.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence presented as SEQ ID NO: 12.

In one preferred embodiment, the isolated polynucleotide of the invention has at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, preferably at least 85%, more preferred at least 90%, more preferred at least 95%, more preferred at least 98% sequence identity to a polynucleotide sequence presented as SEQ ID NO: 18.

A preferred group of isolated polynucleotides include SEQ ID No 1, 2, and 16, which are human NsG33 polynucleotides. Another preferred group of isolated polynucleotides include SEQ ID No. 2, 7, and 12, which represent the cDNA sequences. Generally the cDNA sequence is much shorter than the genomic sequences are are more easily inserted into an appropriate expression vector and transduced/fected into a production cell or a human cell in vivo or ex vivo.

In addition, the nucleotide sequences of the invention include sequences, which are derivatives of these sequences. The invention also includes vectors, liposomes and other carrier vehicles, which encompass one of these sequences or a derivative of one of these sequences. The invention also includes proteins transcribed and translated from NsG33 cDNA, preferably human NsG33 cDNA, including but not limited to human NsG33 and derivatives and variants.

In another embodiment, the invention relates to the use of the nucleic acids and proteins of the present invention to design probes to isolate other genes, which encode proteins with structural or functional properties of the NsG33 proteins of the invention. The probes can be a variety of base pairs in length. For example, a nucleic acid probe can be between about 10 base pairs in length to about 150 base pairs in length.

Alternatively, the nucleic acid probe can be greater than about 150 base pairs in length. Experimental methods are provided in Ausubel et al., "Current Protocols in Molecular Biology", J. Wiley (ed.) (1999), the entire teachings of which are herein incorporated by reference in their entirety.

The design of the oligonucleotide (also referred to herein as nucleic acid) probe should preferably follow these parameters:

i) it should be designed to an area of the sequence which has the fewest ambiguous bases, if any and

ii) it should be designed to have a calculated T_m of about 80°C (assuming 2°C for each A or T and 4°C for each G or C).

The oligonucleotide should preferably be labeled to facilitate detection of hybridisation. Labelling may be with γ-^{32}P ATP (specific activity 6000 Ci/mmole) and T4 polynucleotide kinase using commonly employed techniques for labeling oligonucleotides. Other labeling techniques can also be used. Unincorporated label should preferably be removed by gel filtration chromatography or other established methods. The amount of radioactivity incorporated into the probe should be quantitated by measurement in a scintillation counter. Preferably, specific activity of the resulting probe should be approximately 4 x 10^6 dpm/pmole. The bacterial culture containing the pool of full-length clones should preferably be thawed and 100 µL of the stock used to inoculate a sterile culture flask containing 25 ml of sterile L-broth containing ampicillin at 100 pg/ml.

The culture should preferably be grown to saturation at about 37°C, and the saturated culture should preferably be diluted in fresh L-broth. Aliquots of these dilutions should preferably be plated to determine the dilution and volume which will yield approximately 5000 distinct and well-separated colonies on solid bacteriological media containing L-broth containing ampicillin at 100 pg/ml and agar at 1.5% in a 150 mm petri dish when grown overnight at about 37°C. Other known methods of obtaining distinct, well-separated colonies can also be employed.

Standard colony hybridization procedures should then be used to transfer the colonies to nitrocellulose filters and lyse, denature and bake them. Highly stringent (also referred to herein as "high stringency") conditions are those that are at least as stringent as, for example, 1xSSC at about 65 C, or 1xSSC and 50% formamide at about 42 C. "Moderate stringency" conditions are those that are at least as stringent as 4xSSC at about 65 C, or 4x SSC and 50% formamide at about 42 C. "Reduced stringency" conditions are those that are at least as stringent as 4x SSC at about 50 C, or 6x SSC and 50% formamide at 40 C.

The filter is then preferably incubated at about 65 C for 1 hour with gentle agitation in 6X SSC (20x stock is 175.3 g NaCl/liter, 88.2 g Na citrate/liter, adjusted to pH 7.0 with NaOH) containing 0.5% SDS, 100 g/ml of yeast RNA, and 10 mM EDTA (approximately 10 mL per 150 mm filter). Preferably, the probe is then added to the hybridization mix at a concentration greater than or equal to 1 x 10^6 dpm/mL. The filter is then preferably incubated at about 65 C with gentle agitation overnight. The filter is then preferably washed in 500 mL of 2x SSC/0.5% SDS at room temperature without agitation, preferably followed by 500 mL of 2x SSC/0.1% SDS at room temperature with gentle shaking for 15 minutes. A third wash with 0.1x SSC/0.5% SDS at about 65 C for 30 minutes to 1 hour is optional. The filter is then preferably dried and subjected to autoradiography for sufficient time to visualize the positives on the X-ray film. Other known hybridization methods can also be employed. The positive colonies are then picked, grown in culture, and plasmid DNA isolated using standard procedures. The clones can then be verified by restriction analysis, hybridisation analysis, or DNA sequencing.

Alternatively, suitable experimental conditions for determining hybridization between a nucleotide probe and a homologous DNA or RNA sequence, involves pre-soaking of the filter containing the DNA fragments or RNA to hybridize in 5 x SSC [Sodium chloride/Sodium citrate; cf. *Sambrook et al.*; Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Lab., Cold Spring Harbor, NY 1989] for 10 minutes, and pre-hybridization of the filter in a solution of 5 x SSC, 5 x Denhardt's solution [cf. *Sambrook et al.; Op cit.*], 0.5 % SDS and 100 µg/ml of denatured sonicated salmon sperm DNA [cf. *Sambrook et al.; Op cit.*], followed by hybridization in the same solution containing a concentration of 10 ng/ml of a random-primed [*Feinberg A P & Vogelstein B*; Anal. Biochem. 1983 **132** 6-13], ^{32}P-dCTP-labeled (specific activity > 1 x 10^9 cpm/µg) probe for 12 hours at approximately 45°C. The filter is then washed twice for 30 minutes in 0.1 x SSC, 0.5 % SDS at a temperature of at least at least 60 C (medium stringency conditions), preferably of at least 65 C (medium/high stringency conditions), more preferred of at least 70°C (high stringency conditions), and even more preferred of at least 75°C (very high

stringency conditions). Molecules to which the oligonucleotide probe hybridizes under these conditions may be detected using a x-ray film.

In yet another embodiment, the invention relates to nucleic acid sequences (e. g., DNA, RNA) that hybridise to nucleic acids of NsG33. In particular, nucleic acids which hybridise to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO:6, SEQ ID NO:7, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No 16, SEQ ID No 17, or SEQ ID No 18 under high, moderate or reduced stringency conditions as described above.

In still another embodiment, the invention relates to a complement of nucleic acid of NsG33. In particular, it relates to complements of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID No. 7, SEQ ID No 11, SEQ ID NO 12, SEQ ID No 16, SEQ ID No 17, and SEQ ID No 18.

In another embodiment, the invention relates to an RNA counterpart of the DNA nucleic acid of NsG33. In particular, it relates to RNA counterparts of SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 12, SEQ ID No 16, SEQ ID No 17, and SEQ ID No 18.

Codon optimised nulcleic acid molecules for enhanced expression in selected host cells, including but not limited to E. coli, yeast species, Chinese Hamster, Baby Hamster, insect, and fungus are also contemplated.

Variant nucleic acids can be made by state of the art mutagenesis methods. Methods for shuffling coding sequences from human with those of mouse, rat or chimpanzee are also contemplated. Specifically a shuffled variant may be between SEQ ID No 2 on one hand and 7 and/or 12 on the other hand. Also included are shuffled variants between SEQ ID No 7 and 12.

III USE OF NsG33 POLYPEPTIDES, POLYNUCLEOTIDES, AND NsG33 SECRETING CELLS FOR TREATMENT OF DISORDERS OF THE NERVOUS SYSTEM

In one embodiment, native, variant NsG33, and fragments thereof and/or fusion proteins comprising NsG33 are provided for the treatment of disorders of the mammalian nervous system. NsG33 may be used to stimulate neural cell growth including proliferation, neural function, neural regeneration, neural differentiation, neural migration, and/or neural survival in disease situations where these cells are lost or damaged.

In one embodiment, polynucleotides and/or polypeptides of the invention may be used to treat conditions or diseases where neural growth including proliferation, differentiation, function,

survival, and/or regeneration is desirable. The polypeptides of the present invention may be used directly via, e.g., injected, implanted or ingested pharmaceutical compositions to treat a pathological process responsive to the NsG33 polypeptides. This is supported by the bioinformatics analyses showing that NsG33 is a secreted growth factor and the fact that NsG33 is preferentially expressed in the nervous system, including the eye (Fig. 4).

NsG33 may act on a range of different cell types, which are present in the nervous system. In the context of the present invention, the nervous system is intended to encompass the central nervous system, the peripheral nervous system, the eye, and the cochleovestibular complex.

In one embodiment, NsG33 polypeptides may act on neurons, including but not limited to motor neurons and sensory neurons.

In another embodiment, the therapeutic effect of NsG33 polypeptides may be through action on glial cells, such as oligodendrocytes and/or astrocytes. Through their action on glial cells, NsG33 polypeptides may be involved in myelination, and in the maintenance of neuron function and survival.

In another embodiment, NsG33 polypeptides may act on sensory cells, including but not limited to retinal ganglion cells, photoreceptor cells, supportive tissue such as retinal epithelial cells, and hair cells of the ear.

In a further embodiment, NsG33 polypeptides may act on stem cells, and downstream precursor cells including but not limited to neuronal precursors and glial precursors. NsG33 polypeptides may act on stem cells and/or neuronal or glial precursors to cause growth including proliferation, to cause differentiation, and/or migration. Stem cell therapy may be done through in vivo or ex vivo gene therapy, or the protein may be administered to a location with stem cells.

The disorder or disease or damage may be damages of the nervous system caused by trauma, surgery, ischaemia, infection, metabolic diseases, nutritional deficiency, malignancy or toxic agents, and genetic or idiopathic processes.

In one embodiment of the method of the invention, the disease or disorder or damage involves injury to the brain, brain stem, the spinal cord, and/or peripheral nerves, resulting in conditions such as stroke, traumatic brain injury (TBI), spinal cord injury (SCI), diffuse axonal injury (DAI), epilepsy, neuropathy, peripheral neuropathy, and associated pain and other symptoms that these syndromes may cause.

In another embodiment, the disease, disorder, or damage involves the degeneration of neurons and their processes in the brain, brain stem, the spinal cord, and/or peripheral nerves, such as neurodegenerative disorders including but not limited to Parkinson's Disease, Alzheimer's Disease, senile dementia, Huntington's Disease, amyotrophic lateral sclerosis

(ALS), neuronal/axonal injury associated with Multiple Sclerosis (MS), and associated symptoms.

In another embodiment, the disease, disorder, or damage involves dysfunction, and/or loss of neurons in the brain, brain stem, the spinal cord, and/or peripheral nerves, such as dysfunction and/or loss caused by metabolic diseases, nutritional deficiency, toxic injury, malignancy, and/or genetic or idiopathic conditions, including but not limited to diabetes, renal dysfunction, alcoholism, chemotherapy, chemical agents, drug abuse, vitamin deficiencies, infection, and associated symptoms.

In another embodiment, the disease, disorder, or damage involves the degeneration or sclerosis of glia such as oligodendrocytes, astrocytes, and Schwann cells in the brain, brain stem, the spinal cord, and peripheral nervous system, including but not limited to Multiple Sclerosis (MS), optic neuritis, cerebral sclerosis, post-infectious encephalomyelitis, and epilepsy, and associated symptoms.

In another embodiment, the disease, disorder, or damage involves the retina, photoreceptors, and associated nerves including but not limited to retinitis pigmentosa, macular degeneration, glaucoma, and associated symptoms.

In another embodiment, the disease, disorder, or damage involves the sensory epithelium and associated ganglia of the vestibuloacoustic complex, including but not limited to noise induced hearing loss, deafness, tinnitus, otitis, labyrintitis, hereditary and cochleovestibular atrophies, Meniere's Disease, and associated symptoms.

In another preferred embodiment, the polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of Parkinson's Disease. This function is based on the finding of high levels of expression in the central midbrain in substantia nigra and the putamen (see Example 5) and the finding of expression in the mesencephalon during human embryo development (Example 3). The function can be verified using the Bioassay for dopaminergic neurotrophic activitites (example 11) and in vivo through the instrastriatal 6-OHDA lesion model (Example 12.

Huntington's disease (HD) is an autosomal dominant disorder that results in the progressive degeneration of various neuronal populations within the brain, particularly the GABA-ergic medium spiny neurons located in the caudate nucleus. Associated with this degeneration, the cortical glutaminergic input neurons also degenerate and the combined degeneration account for most of the characteristic symptoms of progressive dyskinetic motor movements as well as dementia.

In a preferred embodiment, the polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of Huntington's disease. This is based on the finding of high expression in the putamen combined

with the results of the bioinformatics analyses. Huntington's disease is an excitotoxic disease. An excitotoxic bioassay is the assay described in Example 12 of the present invention. Another exemplary bioassay for verification of this neuroprotective effect of NsG33 include e.g. the bioassay on protection of primary hippocampal slice cultures against the excitoxic effects of NMDA (WO 03/004527, example 5).

In another preferred embodiment, the polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of peripheral neuropathies. This is based on the finding of high expression in the dorsal root ganglion combined with the results of the bioinformatics analyses. Verification of this function can be done with the dorsal root ganglion culture assay described in example 9. Among the peripheral neuropathies contemplated for treatment with the molecules of this invention are trauma-induced neuropathies, e.g., those caused by physical injury or disease state, physical damage to the peripheral nerves such as hermited discs, and the brain, physical damage to the spinal cord, stroke associated with brain damage, and neurological disorders related to neurodegeneration. We also contemplate treatment of chemotherapy-induced neuropathies (such as those caused by delivery of chemotherapeutic agents, e.g., taxol or cisplatin); toxin-induced neuropathies, drug-induced neuropathies, vitamin-deficiency-induced neuropathies; idiopathic neuropathies; and diabetic neuropathies.

In another preferred embodiment, the polypeptides, nucleic acids, expression vectors, capsules, and compositions of the invention are used in the treatment of disorders, diseases, or damages associated with the Cerebellum, including but not limited to sensory ataxia, multiple sclerosis, neurodegenerative spinocerebellar disorders, hereditary ataxia, cerebellar atrophies (such as Olivopontocerebellar Atrophy (OPCA), Shy-Drager Syndrome (multiple systems atrophy)), and alcoholism. This function is supported by the high expression levels in the cerebellum combined with the bioinformatics analyses. Verification of this function may be done with the assays described in Examples 7 and 8 (Protection of cerebellar granule cells from glutamate toxicity and potassium deprivation).

In another preferred embodiment, the polypeptides, nucleic acids, expression vectors, capsules and pharmaceutical compositions of the invention are used in the treatment of amyotrophic lateral sclerosis, spinal muscular atrophy, and spinal cord injury. This is based on the finding of high expression levels in the spinal cord combined with the results of the bioinformatics analyses. Verification of this specific therapeutic function may be done with the motorneuron assay described in example 10.

In a preferred embodiment, the polypeptides, nucleic acids, vectors, capsules, and compositions of the invention are used in the treatment of diseases, disorders, or damages involving the retina, including but not limited to retinitis pigmentosa, macular degeneration and

glaucoma. This specific therapeutic use is supported by the bioinformatics and experimental analyses showing that NsG33 is a secreted growth factor highly expressed in the retina (Fig. 4).

Other growth factors have important therapeutic uses in both the central and peripheral nervous system and in various eye indications associated with loss of cells in retina and/or cornea. E.g NGF, is a candidate for both Alzheimer's disease, corneal ulcer (US 6,063,757 and EP 0 973 872), and retinopathies. Neublastin (Artemin) is a candidate for both peripheral neuropathy (WO 02/078730) and corneal wound healing (EP 1 223 966). GDNF is a candidate for Parkinson's Disease, ALS, spinal cord injury, and for wound healing, in particular in cornea (EP 1 223 966).

Confirmation of such use can be obtained by using various state of the art in vitro assays (retinal explant assays, corneal cultures). Verfication of function may also be performed in state of the art animal models for corneal wounds (corneal lesion in rabbits) and retina (retinitis pigmentosa mutant models available for mouse and rat).

In another embodiment the neurodegenerative disease is an excitotoxic disease selected from the group consisting of ischaemia, epilepsy, and trauma due to injury, cardiac arrest or stroke. The above-mentioned hippocampal slice culture assay and the assay of Example 7 of the present invention are non-limiting examples of an assay, which can be used to demonstrate a biological effect, indicative of therapeutic use for the treatment of excitotoxic diseases.

The term "subject" used herein is taken to mean any mammal to which NsG33 polypeptide or polynucleotide, therapeutic cells or biocompatible capsules may be administered. Subjects specifically intended for treatment with the method of the invention include humans, as well as nonhuman primates, sheep, horses, cattle, goats, pigs, dogs, cats, rabbits, guinea pigs, hamsters, gerbils, rats and mice, as well as the organs, tumors, and cells derived or originating from these hosts.

IV TREATMENT OF IMMUNOLOGICAL DISORDERS

In one embodiment, NsG33 is contemplated for use in treating immunological disorders. This particular function of NsG33 is based on the structural similarity of NsG33 to a protein with immunological functions described in WO 93/22437 as described above.

According to this embodiment, NsG33 may exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. NsG33 may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e. g., in regulating (up or down) growth and proliferation of

T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e. g. HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using NsG33, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, Leishmania spp., Malaria spp. and various fungal infections such as candidiasis. In this regard, NsG33 may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using NsG33 include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. NsG33 protein (or antagonists thereof, including antibodies) may also to be useful in the treatment of allergic reactions and conditions (e.g., anaphylaxis, serum sickness, drug reactions, food allergies, insect venom allergies, mastocytosis, allergic rhinitis, hypersensitivity pneumonitis, urticaria, angioedema, eczema, atopic dermatitis, allergic contact dennatitis, erythema multiforme, Stevens-Johnson syndrome, allergic conjunctivitis, atopic keratoconjunctivitis, venereal keratoconjunctivitis, giant papillary conjunctivitis and contact allergies), such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using an NsG33 protein (or antagonists thereof). The therapeutic effects of the polypeptides or antagonists thereof on allergic reactions can be evaluated by in vivo animals models such as the cumulative contact enhancement test (Lastbom et al. , Toxicology 125: 59-66,1998), skin prick test (Hoffmann et al., Allergy 54: 446-54,1999), guinea pig skin sensitization test (Vohr et al. , Arch. Toxocol. 73: 501-9), and murine local lymph node assay (Kimber et al., J. Toxicol. Environ. Health 53: 563-79).

Using NsG33 it may also be possible to modulate immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is

distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased.

Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as, for example, B7)), e. g. preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation.

Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a pharmaceutical composition of the invention may prevent cytokine synthesis by immune cells, such as T cells, and thus acts as animmunosuppressant. Moreover, a lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular pharmaceutical compositions in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins in vivo as described in Lenschow et al. , Science 257: 789-792 (1992) and Turka et al., Proc. Natl. Acad. Sci USA, 89: 11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of therapeutic compositions of the invention on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self-tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or

eliminate disease symptoms. Administration of reagents which block stimulation of T cells can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (e. g. a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response may be useful in cases of viral infection, including systemic viral diseases such as influenza, the common cold, and encephalitis.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells in vitro with viral antigen-pulsed APCs either expressing an NsG33 or together with a stimulatory form of a soluble NsG33 of the present invention and reintroducing the in vitro activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding NsG33 as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells in vivo.

NsG33 may provide the necessary stimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e. g. a cytoplasmic-domain truncated portion) of an MHC class I alpha chain protein and $_2$ microglobulin protein or an MHC class II alpha chain protein and an MHC class II beta chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression

of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (e. g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of NsG33 may, among other means, be measured by the following methods:
Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3. 19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78: 2488-2492,1981; Herrmann et al., J. Immunol. 128: 1968-1974,1982; Handa et al., J. Immunol. 135: 1564-1572,1985; Takai et al., I. Immunol. 137: 3494-3500,1986; Takai et al., J. Immunol. 140: 508-512,1988; Bowman et al., J. Virology 61: 1992-1998; Bertagnolli et al., Cellular Immunology 133: 327-341,1991; Brown et al. , J. Immunol. 153: 3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Thl/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144: 3028-3033,1990; and Assays for B cell function: In vitro antibody production, Mond, J. J. and Brunswick, M. In Current Protocols in Immunology. J. E. e. a. Coligan eds. Vol 1 pp. 3.8. 1-3.8. 16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3. 19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137: 3494-3500,1986; Takai et al. , J. Immunol. 140: 508-512,1988; Bertagnolli et al. , J. Immunol. 149: 3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134: 536-544,1995; Inaba et al., Journal of Experimental Medicine 173: 549-559,1991; Macatonia et al., Journal of Immunology 154: 5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182: 255-260, 1995; Nair et al., Journal of Virology 67: 4062-4069,1993; Huang et al., Science 264: 961-965,1994; Macatonia et al., Journal of Experimental Medicine 169: 1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172: 631-640,1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13: 795-808,1992; Gorczyca et al., Leukemia 7: 659-670,1993; Gorczyca et al., Cancer Research 53: 1945-1951, 1993; Itoh et al., Cell 66: 233-243, 1991; Zacharchuk, Journal of Immunology 145: 4037-4045, 1990; Zamai et al., Cytometry 14: 891-897, 1993; Gorczyca et al., International Journal of Oncology 1: 639-648,1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84: 111-117, 1994 ; Fine et al., Cellular Immunology 155: 111-122, 1994; Galy et al., Blood 85: 2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88: 7548-7551, 1991.

V. Polypeptide Administration and Formulations

A target tissue for NsG33 therapy is a region of the brain is selected for its retained responsiveness to NsG33. In humans, neurons, which retain responsiveness to growth factors into adulthood include the cholinergic basal forebrain neurons, the entorhinal cortical neurons, the thalamic neurons, the locus coeruleus neurons, the spinal sensory neurons, the spinal motor neurons, neurons of substantia nigra, sympathetic neurons, dorsal root ganglia, retina neurons, otic neurons, cerebellar neurons, and ciliary ganglia. Stem cells, such as stem cells of the subventricular zone, and neural and glial progenitor cells also retain responsiveness to growth factors into adulthood. Also myelinating oligodendrocytes retain responsiveness to growth factors into adulthood.

NsG33 polypeptides may be administered in any manner, which is medically acceptable. This may include injections, by parenteral routes such as intravenous, intravascular, intraarterial, subcutaneous, intramuscular, intratumor, intraperitoneal, intraventricular,

intraepidural, intertracheal, intrathecal, intracerebroventricular, intercerebral, interpulmonary, or others as well as nasal, ophthalmic, rectal, or topical. Sustained release administration is also specifically included in the invention, by such means as depot injections or erodible implants. Peroral administration is also conceivable provided the protein is protected against degradation in the stomach.

Administration of an NsG33 according to this invention may be achieved using any suitable delivery means, including:

pump (see, e.g., Annals of Pharmacotherapy, 27:912 (1993); Cancer, 41:1270 (1993); Cancer Research, 44:1698 (1984), incorporated herein by reference),

microencapsulation (see, e.g., United States patents 4,352,883; 4,353,888; and 5,084,350, herein incorporated by reference),

continuous release polymer implants (see, e.g., Sabel, United States patent 4,883,666, incorporated herein by reference),

encapsulated cells (see, Section X),

naked or unencapsulated cell grafts to the CNS (see, e.g., United States patents 5,082,670 and 5,618,531, each incorporated herein by reference);

injection, either subcutaneously, intravenously, intra-arterially, intramuscularly, or to other suitable site;

inhalation; and

oral administration, in capsule, liquid, tablet, pill, or prolonged release formulation.

Administration may be by periodic injections of a bolus of the preparation, or may be made more continuous by intravenous or intraperitoneal administration from a reservoir which is external (*e.g.*, an IV bag) or internal (*e.g.*, a bioerodable implant, a bioartificial organ, a biocompatible capsule of NsG33 production cells, or a colony of implanted NsG33 production cells). See, *e.g.*, U.S. Patents 4,407,957, 5,798,113, and 5,800,828, each incorporated herein by reference. Intrapulmonary delivery methods and apparatus are described, for example, in U.S. Patents 5,654,007, 5,780,014, and 5,814,607, each incorporated herein by reference.

Apart from systemic delivery, delivery directly to the CNS or the eye behind the blood-brain or blood-retina barriers is also contemplated.

Localised delivery may be by such means as delivery via a catheter to one or more arteries, such as the ophthalmic artery to the eye, and the cerebral artery to the CNS. Methods for local pump-based delivery of protein formulations to the CNS are described in US 6,042,579 (Medtronic). Another type of localised delivery comprises delivery using encapsulated cells (see Section X). A further type of localised delivery comprises local delivery of gene therapy vectors, which are normally injected.

For the treatment of eye disorders, delivery may be systemic, or local such as delivery via the ophthalmic artery. In another embodiment, delivery is via Encapsulated Cell Therapy, where the encapsulated cells are implanted intravitreally. Delivery of protein formulations or gene therapy vector may be done using subretinal injections, intravitreal injection, or transcleral injection.

For the treatment of Parkinson's Disease, various delivery routes can be taken. Protein formulations can be administred with pumps intracerbroventricularly or intraparenchymally, preferably to the striatum and/or substantia nigra, more preferably to the intraputamen. However, a more preferred delivery method comprises encapsulated cell therapy, where the capsulses are implanted intracerebroventricularly, or intraparenchymally, preferably into the striatum, and/or substantia nigra, and more preferably into the putamen. In one embodiment relating to treatment of Parkinson's Disease, gene therapy vector is administered to the striatum of the brain. Injection into the striatum can label target sites located in various distant regions of the brain, for example, the globus pallidus, amygdala, subthalamic nucleus or the substantia nigra. Transduction of cells in the pallidus commonly causes retrograde labelling of cells in the thalamus. In a preferred embodiment the (or one of the) target site(s) is the substantia nigra.

In an embodiment to treat HD, NsG33 is applied to the striatum, preferably the caudate nucleus in order to protect the neurons from degeneration, resulting in both protection of the caudate neurons and the cortical input neurons. In a preferred embodiment, the application should occur before the onset of major degenerative changes. The treatment would involve the genetic diagnosis of the disease through family history and DNA analysis of the blood followed by the local application of NsG-33. This would be accomplished by delivering the NsG33 to the striatum via pumping of the protein with the use of medically applicable infusion pumps and catheters, e.g. Medtronic Synchrotron pump. In a second strategy, direct gene therapy using viral or non-viral vectors could be utilized to modify the host cells in the striatum or other affected neurons to secrete NsG33. In a third strategy, naked or encapsulated cells genetically modified to make and secrete NsG33 can be applied locally to deliver NsG33 behind the blood-brain-barrier and within the diseased region, preferably the striatum, even more preferred, the caudate nucleus.

In ALS, both upper and lower motor neurons degenerate, causing progressive paralyses, eventually leading to death, most commonly through respiratory complications. To treat ALS, NsG33 would be delivered to the CNS including the spinal cord through the infusion of NsG33 into the lumbar intrathecal space thereby mixing with the cerebrospinal fluid (CSF), which bathes the spinal cord and brain. The delivery could be accomplished through the implantation of pump and catheters, e.g. Medtronic Synchrotron pump or through the use of encapsulated cell devices implanted into the lumbar inthrathecal space. Direct gene therapy

could also be used by injecting DNA carrying vectors into the CSF, thereby transferring the gene to cells lining the CSF space. In addition, gene transfer vectors can be injected into the cervical or lumbar spinal cord or intracerebral, thereby secreting NsG33 in the anatomical regions containing the majority of the motor neurons involved in motor paralyses and respiratory function. These injections would occur under surgical navigation and could be performed relatively safely.

In subjects with neurodegenerative diseases such as AD, neurons in the Ch4 region (nucleus basalis of Meynert) which have nerve growth factor (NGF) receptors undergo marked atrophy as compared to normal controls (see, e. g., Kobayashi, et al., Mol. Chem. Neuropathol., 15: 193-206 (1991)).

In normal subjects, neurotrophins prevent sympathetic and sensory neuronal death during development and prevents cholinergic neuronal degeneration in adult rats and primates (Tuszynski, et al., Gene Therapy, 3 : 305314 (1996)). The resulting loss of functioning neurons in this region of the basal forebrain is believed to be causatively linked to the cognitive decline experienced by subjects suffering from neurodegenerative conditions such as AD (Tuszynski, et al., supra and, Lehericy, et al., J. Comp. Neurol., 330: 15-31 (1993)).

In general it is contemplated, that AD can be treated with NsG33 protein formulations delivered intracerebroventricularly, or intraparenchymally. Within the intraparenchymal area, delivery is preferably to the basal forebrain, and to the hippocampus.

Gene therapy vector, encapsulated or naked cells secreting NsG33 can also be administered to the basal forebrain or the hippocampus.

For the treatment of spinal cord injury, protein, gene therapy vector or encapsulated or naked cells secreting NsG33 can be delivered intrathecally at the position of the injury as described above for the treatment of ALS.

For the treatment of peripheral neuropathy, delivery is either systemic (using protein formulations), intrathecally using protein formulations, gene therapy vectors, or encapsulated or naked cells secreting NsG33, or intramuscularly depending on retrograde transport to the spinal cord.

For the treatment of epilepsy NsG33 protein could be delivered intraparenchymally in the epilepsy focus. This may be done with encapsulated or naked cells, with protein formulation administered with catheter or pump or with gene therapy vector delivered to this site.

For the treatment of stroke or trauma, delivery is intrathecal, intracerbroventricular, or preferably intralessionar.

The term "pharmaceutically acceptable carrier" means one or more organic or inorganic ingredients, natural or synthetic, with which NsG33 polypeptide is combined to facilitate its application. A suitable carrier includes sterile saline although other aqueous and non-aqueous

isotonic sterile solutions and sterile suspensions known to be pharmaceutically acceptable are known to those of ordinary skill in the art. An "effective amount" refers to that amount which is capable of ameliorating or delaying progression of the diseased, degenerative or damaged condition. An effective amount can be determined on an individual basis and will be based, in part, on consideration of the symptoms to be treated and results sought. An effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

The liposome system may be any variety of unilamellar vesicles, multilamellar vesicles, or stable plurilamellar vesicles, and may be prepared and administered according to methods well known to those of skill in the art, for example in accordance with the teachings of United States Patents 5,169,637,4,762,915, 5,000,958 or 5,185,154. In addition, it may be desirable to express the novel polypeptides of this invention, as well as other selected polypeptides, as lipoproteins, in order to enhance their binding to liposomes. A recombinant NsG33 protein is purified, for example, from CHO cells by immunoaffinity chromatography or any other convenient method, then mixed with liposomes and incorporated into them at high efficiency. The liposome-encapsulated protein may be tested in vitro for any effect on stimulating cell growth.

Any of the NsG33 polypeptides of this invention may be used in the form of a pharmaceutically acceptable salt. Suitable acids and bases which are capable of forming salts with an NsG33 polypeptide are well known to those of skill in the art, and include inorganic and organic acids and bases.

In addition to the active ingredients, the pharmaceutical compositions may comprise suitable ingredients. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

Various dosing regimes for systemic administration are contemplated. In one embodiment, methods of administering to a subject a formulation comprising an NsG33 polypeptide include administering NsG33 at a dosage of between 1 µg/kg to 30,000 µg/kg body weight of the subject, per dose. In another embodiment, the dosage is between 10 µg/kg to 30,000 µg/kg body weight of the subject, per dose. In a further embodiment, the dosage is between 10 µg/kg to 10,000 µg/kg body weight of the subject, per dose. In a different embodiment, the dosage is between 25 µg/kg to 10,000 µg/kg body weight of the subject, per dose. In yet another embodiment, the dosage is between 25 µg/kg to 3,000 µg/kg body weight of the subject, per dose. In a most preferable embodiment, the dosage is between 50 µg/kg to 3,000 µg/kg body weight of the subject, per dose.

Guidance as to particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Pat. Nos. 4,657,760; 5,206,344; or 5,225,212. It is anticipated that different formulations will be effective for different treatment compounds and different disorders, that administration targeting one organ or tissue, for example, may necessitate delivery in a manner different from that to another organ or tissue.

Where sustained-release administration of an NsG33 polypeptide is desired in a formulation with release characteristics suitable for the treatment of any disease or disorder requiring administration of an NsG33 polypeptide, microencapsulation of an NsG33 polypeptide is contemplated. Microencapsulation of recombinant proteins for sustained release has been successfully performed with human growth hormone (rhGH), interferon-(rhIFN-), interleukin-2, and MN rgp120. Johnson et al., Nat. Med., 2:795-799 (1996); Yasuda, Biomed. Ther., 27:1221-1223 (1993); Hora et al., Bio/Technology, 8:755-758 (1990); Cleland, "Design and Production of Single Immunization Vaccines Using Polylactide Polyglycolide Microsphere Systems," in Vaccine Design: The Subunit and Adjuvant Approach, Powell and Newman, eds, (Plenum Press: New York, 1995), pp. 439-462; WO 97/03692, WO 96/40072, WO 96/07399; and U.S. Pat. No. 5,654,010.

The sustained-release formulations of these proteins were developed using poly-lactic-coglycolic acid (PLGA) polymer due to its biocompatibility and wide range of biodegradable properties. The degradation products of PLGA, lactic and glycolic acids, can be cleared quickly within the human body. Moreover, the degradability of this polymer can be adjusted from months to years depending on its molecular weight and composition. Lewis, "Controlled release of bioactive agents from lactide/glycolide polymer," in: M. Chasin and R. Langer (Eds.), Biodegradable Polymers as Drug Delivery Systems (Marcel Dekker: New York, 1990), pp. 1-41.

The dose administered must be carefully adjusted to the age, weight and condition of the individual being treated, as well as the route of administration, dosage form and regimen, and the result desired, and the exact dosage should be determined by the practitioner.

VI. Pharmaceutical preparations for gene therapy

To form an NsG33 composition for gene therapy use in the invention, NsG33 encoding expression viral vectors may be placed into a pharmaceutically acceptable suspension, solution or emulsion. Suitable mediums include saline and liposomal preparations.

More specifically, pharmaceutically acceptable carriers may include sterile aqueous of non-aqueous solutions, suspensions, and emulsions. Examples of nonaqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic

esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils.

Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like.

Preservatives and other additives may also be present such as, for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like. Further, a composition of NsG33 transgenes may be lyophilized using means well known in the art, for subsequent reconstitution and use according to the invention.

A colloidal dispersion system may also be used for targeted gene delivery. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposoms. Liposomes are artificial membrane vesicles which are useful as delivery vehicles in vitro and in vivo. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 μm can encapsulate a substantial percentage of an aqueous buffer containing large macro molecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., Trends Biochem. Sci., 6: 77,1981). In addition to mammalian cells, liposomes have been used for delivery of operatively encoding transgenes in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes encoding the NsG33 at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, et al., Biotechniques, 6: 682,1988).

The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries.

Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

The surface of the targeted gene delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand.

A further example of a delivery system includes transplantation into the therapeutic area of a composition of packaging cells capable of producing vector particles as described in the present invention. Methods for encapsulation and transplantation of such cells are known in the art, in particular from WO 97/44065 (Cytotherapeutics). By selecting a packaging cell line capable of producing lentiviral particles, transduction of non-dividing cells in the therapeutic area is obtained. By using retroviral particles capable of transducing only dividing cells, transduction is restricted to de-novo differentiated cells in the therapeutic area.

VII. Dosing Requirements and Delivery Protocol for gene therapy

An important parameter is the dosage of NsG33 gene therapy vector to be delivered into the target tissue. For viral vectors, the concentration may be defined by the number of transducing

units/ml. Optimally, for delivery using a viral expression vector, each unit dosage will comprise 2.5 to 25 µL of a composition, wherein the composition includes a viral expression vector in a pharmaceutically acceptable fluid and provides from 10^8 up to 10^{10} NsG33 transducing units per ml.

Importantly, specific in vivo gene delivery sites are selected so as to cluster in an area of loss, damage, or dysfunction of neural cells, glial cells, retinal cells, sensory cells, or stem cells. Such areas may be identified clinically using a number of known techniques, including magnetic resonance imaging (MRI) and biopsy. In humans, non-invasive, in vivo imaging methods such as MRI will be preferred. Once areas of neuronal loss are identified, delivery sites are selected for stereotaxic distribution so each unit dosage of NsG33 is delivered into the brain at, or within 500 µm from, a targeted cell, and no more than about 10 mm from another delivery site.

Within a given target site, the vector system may transduce a target cell. The target cell may be a cell found in nervous tissue, such as a neuron, astrocyte, oligodendrocyte, microglia, stem cells, neural precursor cells, or ependymal cell.

The vector system is preferably administered by direct injection. Methods for injection into the brain are well known in the art (Bilang-Bleuel et al (1997) Proc. Acad. Nati. Sci. USA 94:8818-8823; Choi-Lundberg et al (1998) Exp. Neurol.154:261-275; Choi-Lundberg et al (1997) Science 275:838-841; and Mandel et al (1997)) Proc. Acad. Natl. Sci. USA 94:14083-14088). Stereotaxic injections may be given.

As mentioned above, for transduction in tissues such as the brain, it is necessary to use very small volumes, so the viral preparation is concentrated by ultracentrifugation. The resulting preparation should have at least 10^8 t.u./ml, preferably from 10^8 to 10^{10} t.u./ml, more preferably at least 10^9 t.u./ml. (The titer is expressed in transducing units per ml (t.u./ml) as described in example 6). It has been found that improved dispersion of transgene expression can be obtained by increasing the number of injection sites and decreasing the rate of injection (Horellou and Mallet (1997) as above). Usually between 1 and 10 injection sites are used, more commonly between 2 and 6. For a dose comprising 1-5 10^9 t.u./ml, the rate of injection is commonly between 0.1 and 10 µl/min, usually about 1 µl/min.

The virus composition is delivered to each delivery cell site in the target tissue by microinjection, infusion, scrape loading, electroporation or other means suitable to directly deliver the composition directly into the delivery site tissue through a surgical incision. The delivery is

accomplished slowly, such as over a period of about 5-10 minutes (depending on the total volume of virus composition to be delivered).

VIII. Viral vectors

Broadly, gene therapy seeks to transfer new genetic material to the cells of a patient with resulting therapeutic benefit to the patient. Such benefits include treatment or prophylaxis of a broad range of diseases, disorders and other conditions.

Ex vivo gene therapy approaches involve modification of isolated cells (including but not limited to stem cells, neural and glial precursor cells, and foetal stem cells), , which are then infused, grafted or otherwise transplanted into the patient. See, e.g., U.S. Pat. Nos. 4,868,116, 5,399,346 and 5,460,959. In vivo gene therapy seeks to directly target host patient tissue in vivo.

Viruses useful as gene transfer vectors include papovavirus, adenovirus, vaccinia virus, adeno-associated virus, herpesvirus, and retroviruses. Suitable retroviruses include the group consisting of HIV, SIV, FIV, EIAV, MoMLV.

Preferred viruses for treatment of disorders of the nervous system are lentiviruses and adeno-associated viruses. Both types of viruses can integrate into the genome without cell divisions, and both types have been tested in pre-clinical animal studies for indiations of the nervous system, in particular the central nervous system.

Methods for preparation of AAV are described in the art, e.g. US 5,677,158. US 6,309,634 and US 6,683,058 describe examples of delivery of AAV to the central nervous system.

Preferably, a lentivirus vector is a replication-defective lentivirus particle. Such a lentivirus particle can be produced from a lentiviral vector comprising a 5' lentiviral LTR, a tRNA binding site, a packaging signal, a promoter operably linked to a polynucleotide signal encoding said fusion protein, an origin of second strand DNA synthesis and a 3' lentiviral LTR. Methods for preparation and in vivo administration of lentivirus to neural cells are described in US 20020037281 (Methods for transducing neural cells using lentiviral vectors).

Retroviral vectors are the vectors most commonly used in human clinical trials, since they carry 7-8 kb and since they have the ability to infect cells and have their genetic material stably

integrated into the host cell with high efficiency. See, e.g., WO 95/30761; WO 95/24929. Oncovirinae require at least one round of target cell proliferation for transfer and integration of exogenous nucleic acid sequences into the patient. Retroviral vectors integrate randomly into the patient's genome. Retroviruses can be used to target stem cells of the nervous system as very few cell divisions take place in other cells of the nervous system (in particular the CNS).

Three classes of retroviral particles have been described; ecotropic, which can infect murine cells efficiently, and amphotropic, which can infect cells of many species. The third class includes xenotrophic retrovirus which can infect cells of another species than the species which produced the virus. Their ability to integrate only into the genome of dividing cells has made retroviruses attractive for marking cell lineages in developmental studies and for delivering therapeutic or suicide genes to cancers or tumors.

For use in human patients, the retroviral vectors must be replication defective. This prevents further generation of infectious retroviral particles in the target tissue--instead the replication defective vector becomes a "captive" transgene stable incorporated into the target cell genome. Typically in replication defective vectors, the gag, env, and pol genes have been deleted (along with most of the rest of the viral genome). Heterologous DNA is inserted in place of the deleted viral genes. The heterologous genes may be under the control of the endogenous heterologous promoter, another heterologous promoter active in the target cell, or the retroviral 5' LTR (the viral LTR is active in diverse tissues). Typically, retroviral vectors have a transgene capacity of about 7-8 kb.

Replication defective retroviral vectors require provision of the viral proteins necessary for replication and assembly in trans, from, e.g., engineered packaging cell lines. It is important that the packaging cells do not release replication competent virus and/or helper virus. This has been achieved by expressing viral proteins from RNAs lacking the signal, and expressing the gag/pol genes and the env gene from separate transcriptional units. In addition, in some 2. and 3. generation retriviruses, the 5' LTR's have been replaced with non-viral promoters controlling the expression of these genes, and the 3' promoter has been minimised to contain only the proximal promoter. These designs minimize the possibility of recombination leading to production of replication competent vectors, or helper viruses.

IX. Expression vectors

Construction of vectors for recombinant expression of NsG33 polypeptides for use in the invention may be accomplished using conventional techniques which do not require detailed explanation to one of ordinary skill in the art. For review, however, those of ordinary skill may wish to consult Maniatis et al., in Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, (NY 1982). Expression vectors may be used for generating producer cells for recombinant production of NsG33 polypeptides for medical use, and for generating therapeutic cells secreting NsG33 polypeptides for naked or encapsulated therapy.

Briefly, construction of recombinant expression vectors employs standard ligation techniques. For analysis to confirm correct sequences in vectors constructed, the genes are sequenced using, for example, the method of Messing, et al., (Nucleic Acids Res., 9: 309-, 1981), the method of Maxam, et al., (Methods in Enzymology, 65: 499, 1980), or other suitable methods which will be known to those skilled in the art.

Size separation of cleaved fragments is performed using conventional gel electrophoresis as described, for example, by Maniatis, et al., (Molecular Cloning, pp. 133-134,1982).

For generation of efficient expression vectors, these should contain regulatory sequences necessary for expression of the encoded gene in the correct reading frame. Expression of a gene is controlled at the transcription, translation or post-translation levels. Transcription initiation is an early and critical event in gene expression. This depends on the promoter and enhancer sequences and is influenced by specific cellular factors that interact with these sequences. The transcriptional unit of many genes consists of the promoter and in some cases enhancer or regulator elements (Banerji et al., Cell 27: 299 (1981); Corden et al., Science 209: 1406 (1980); and Breathnach and Chambon, Ann. Rev. Biochem. 50: 349 (1981)). For retroviruses, control elements involved in the replication of the retroviral genome reside in the long terminal repeat (LTR) (Weiss et al., eds., The molecular biology of tumor viruses: RNA tumor viruses, Cold Spring Harbor Laboratory, (NY 1982)). Moloney murine leukemia virus (MLV) and Rous sarcoma virus (RSV) LTRs contain promoter and enhancer sequences (Jolly et al., Nucleic Acids Res. 11: 1855 (1983); Capecchi et al., In : Enhancer and eukaryotic gene expression, Gulzman and Shenk, eds., pp. 101-102, Cold Spring Harbor Laboratories (NY 1991). Other potent promoters include those derived from cytomegalovirus (CMV) and other wild-type viral promoters.

Promoter and enhancer regions of a number of non-viral promoters have also been described (Schmidt et al., Nature 314: 285 (1985); Rossi and deCrombrugghe, Proc. Natl. Acad. Sci. USA

84: 5590-5594 (1987)). Methods for maintaining and increasing expression of transgenes in quiescent cells include the use of promoters including collagen type I (1 and 2) (Prockop and Kivirikko, N. Eng. J. Med. 311: 376 (1984) ; Smith and Niles, Biochem. 19: 1820 (1980) ; de Wet et al., J. Biol. Chem., 258: 14385 (1983)), SV40 and LTR promoters.

According to one embodiment of the invention, the promoter is a constitutive promoter selected from the group consisting of: ubiquitin promoter, CMV promoter, JeT promoter (US 6,555,674), SV40 promoter, and Elongation Factor 1 alpha promoter (EF1-alpha).

Examples of inducible/repressible promoters include: Tet-On, Tet-Off, Rapamycin-inducible promoter, Mx1.

In addition to using viral and non-viral promoters to drive transgene expression, an enhancer sequence may be used to increase the level of transgene expression. Enhancers can increase the transcriptional activity not only of their native gene but also of some foreign genes (Armelor, Proc. Natl. Acad. Sci. USA 70 : 2702 (1973)). For example, in the present invention collagen enhancer sequences may be used with the collagen promoter 2 (I) to increase transgene expression. In addition, the enhancer element found in SV40 viruses may be used to increase transgene expression. This enhancer sequence consists of a 72 base pair repeat as described by Gruss et al., Proc. Natl. Acad. Sci. USA 78: 943 (1981); Benoist and Chambon, Nature 290: 304 (1981), and Fromm and Berg, J. Mol. Appl. Genetics, 1 : 457 (1982), all of which are incorporated by reference herein. This repeat sequence can increase the transcription of many different viral and cellular genes when it is present in series with various promoters (Moreau et al., Nucleic Acids Res. 9 : 6047 (1981).

Further expression enhancing sequences include but are not limited to Woodchuck hepatitis virus post-transcriptional regulation element, WPRE, SP163, CMV enhancer, and Chicken [beta]-globin insulator or other insulators.

Transgene expression may also be increased for long term stable expression using cytokines to modulate promoter activity. Several cytokines have been reported to modulate the expression of transgene from collagen 2 (I) and LTR promoters (Chua et al., connective Tissue Res., 25: 161-170 (1990); Elias et al., Annals N. Y. Acad. Sci., 580 : 233-244 (1990)); Seliger et al., J. Immunol. 141: 2138-2144 (1988) and Seliger et al., J. Virology 62: 619-621 (1988)). For example, transforming growth factor (TGF), interleukin (IL)-I, and interferon (INF) down regulate the expression of transgenes driven by various promoters such as LTR. Tumor necrosis factor

(TNF) and TGF 1 up regulate, and may be used to control, expression of transgenes driven by a promoter. Other cytokines that may prove useful include basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF).

Collagen promoter with the collagen enhancer sequence (Coll (E)) may also be used to increase transgene expression by suppressing further any immune response to the vector which may be generated in a treated brain notwithstanding its immune-protected status. In addition, anti-inflammatory agents including steroids, for example dexamethasone, may be administered to the treated host immediately after vector composition delivery and continued, preferably, until any cytokine-mediated inflammatory response subsides. An immunosuppression agent such as cyclosporin may also be administered to reduce the production of interferons, which downregulates LTR promoter and Coll (E) promoter-enhancer, and reduces transgene expression.

The vector may comprise further sequences such as a sequence coding for the Cre-recombinase protein, and LoxP sequences. A further way of ensuring temporary expression of the NsG33 is through the use of the Cre-LoxP system which results in the excision of part of the inserted DNA sequence either upon administration of Cre-recombinase to the cells (Daewoong et al, Nature Biotechnology 19:929-933) or by incorporating a gene coding for the recombinase into the virus construct (Plück, Int J Exp Path, 77:269-278). Incorporating a gene for the recombinase in the virus construct together with the LoxP sites and a structural gene (an NsG33 in the present case) often results in expression of the structural gene for a period of approximately five days.

X. Biocompatible capsules

Encapsulated cell therapy is based on the concept of isolating cells from the recipient host's immune system by surrounding the cells with a semipermeable biocompatible material before implantation within the host. The invention includes a device in which cells capable of expressing and secreting NsG33 are encapsulated in an immunoisolatory capsule. An "immunoisolatory capsule" means that the capsule, upon implantation into a recipient host, minimizes the deleterious effects of the host's immune system on the cells in the core of the device. Cells are immunoisolated from the host by enclosing them within implantable polymeric capsules formed by a microporous membrane. This approach prevents the cell-to cell contact between host and implanted tissues, eliminating antigen recognition through direct presentation. The membranes used can also be tailored to control the diffusion of molecules, such as

antibody and complement, based on their molecular weight (Lysaght et al., 56 J. Cell Biochem. 196 (1996), Colton, 14 Trends Biotechnol. 158 (1996)). Using encapsulation techniques cells can be transplanted into a host without immune rejection, either with or without use of immunosuppressive drugs. Useful biocompatible polymer capsules usually contain a core that contains cells, either suspended in a liquid medium or immobilized within an immobilizing matrix, and a surrounding or peripheral region of permselective matrix or membrane ("jacket") that does not contain isolated cells, that is biocompatible, and that is sufficient to protect cells in the core from detrimental immunological attack. Encapsulation hinders elements of the immune system from entering the capsule, thereby protecting the encapsulated cells from immune destruction. The semipermeable nature of the capsule membrane also permits the biologically active molecule of interest to easily diffuse from the capsule into the surrounding host tissue.

The capsule can be made from a biocompatible material. A "biocompatible material" is a material that, after implantation in a host, does not elicit a detrimental host response sufficient to result in the rejection of the capsule or to render it inoperable, for example through degradation. The biocompatible material is relatively impermeable to large molecules, such as components of the host's immune system, but is permeable to small molecules, such as insulin, growth factors such as NsG33 polypeptides, and nutrients, while allowing metabolic waste to be removed. A variety of biocompatible materials are suitable for delivery of growth factors by the composition of the invention. Numerous biocompatible materials are known, having various outer surface morphologies and other mechanical and structural characteristics. Preferably the capsule of this invention will be similar to those described in WO 92/19195 or WO 95/05452, incorporated by reference; or U.S. Pat. Nos. 5,639,275; 5,653,975; 4,892,538; 5,156,844; 5,283,187; or U.S. Pat. No. 5,550,050, incorporated by reference. Such capsules allow for the passage of metabolites, nutrients and therapeutic substances while minimizing the detrimental effects of the host immune system. Components of the biocompatible material may include a surrounding semipermeable membrane and the internal cell-supporting scaffolding. Preferably, the genetically altered cells are seeded onto the scaffolding, which is encapsulated by the permselective membrane. The filamentous cell-supporting scaffold may be made from any biocompatible material selected from the group consisting of acrylic, polyester, polyethylene, polypropylene polyacetonitrile, polyethylene teraphthalate, nylon, polyamides, polyurethanes, polybutester, silk, cotton, chitin, carbon, or biocompatible metals. Also, bonded fiber structures can be used for cell implantation (U.S. Pat. No. 5,512,600, incorporated by reference). Biodegradable polymers include those comprised of poly(lactic acid) PLA, poly(lactic-coglycolic acid) PLGA, and poly(glycolic acid) PGA and their equivalents. Foam scaffolds have been used to provide surfaces onto which transplanted cells may adhere (WO 98/05304, incorporated by

reference). Woven mesh tubes have been used as vascular grafts (WO 99/52573, incorporated by reference). Additionally, the core can be composed of an immobilizing matrix formed from a hydrogel, which stabilizes the position of the cells. A hydrogel is a 3-dimensional network of cross-linked hydrophilic polymers in the form of a gel, substantially composed of water.

Various polymers and polymer blends can be used to manufacture the surrounding semipermeable membrane, including polyacrylates (including acrylic copolymers), polyvinylidenes, polyvinyl chloride copolymers, polyurethanes, polystyrenes, polyamides, cellulose acetates, cellulose nitrates, polysulfones (including polyether sulfones), polyphosphazenes, polyacrylonitriles, poly(acrylonitrile/covinyl chloride), as well as derivatives, copolymers and mixtures thereof. Preferably, the surrounding semipermeable membrane is a biocompatible semipermeable hollow fiber membrane. Such membranes, and methods of making them are disclosed by U.S. Pat. Nos. 5,284,761 and 5,158,881, incorporated by reference. The surrounding semipermeable membrane is formed from a polyether sulfone hollow fiber, such as those described by U.S. Pat. No. 4,976,859 or U.S. Pat. No. 4,968,733, incorporated by reference. An alternate surrounding semipermeable membrane material is poly(acrylonitrile/covinyl chloride).

The capsule can be any configuration appropriate for maintaining biological activity and providing access for delivery of the product or function, including for example, cylindrical, rectangular, disk-shaped, patch-shaped, ovoid, stellate, or spherical. Moreover, the capsule can be coiled or wrapped into a mesh-like or nested structure. If the capsule is to be retrieved after it is implanted, configurations which tend to lead to migration of the capsules from the site of implantation, such as spherical capsules small enough to travel in the recipient host's blood vessels, are not preferred. Certain shapes, such as rectangles, patches, disks, cylinders, and flat sheets offer greater structural integrity and are preferable where retrieval is desired.

When macrocapsules are used, preferably between 10^3 and 10^8 cells are encapsulated, most preferably 10^5 to 10^7 cells are encapsulated in each device. Dosage may be controlled by implanting a fewer or greater number of capsules, preferably between 1 and 10 capsules per patient.

The scaffolding may be coated with extracellular matrix (ECM) molecules. Suitable examples of extracellular matrix molecules include, for example, collagen, laminin, and fibronectin. The surface of the scaffolding may also be modified by treating with plasma irradiation to impart charge to enhance adhesion of cells.

Any suitable method of sealing the capsules may be used, including the use of polymer adhesives or crimping, knotting and heat sealing. In addition, any suitable "dry" sealing method can also be used, as described, e.g., in U.S. Pat. No. 5,653,687, incorporated by reference.

The encapsulated cell devices are implanted according to known techniques. Many implantation sites are contemplated for the devices and methods of this invention. These implantation sites include, but are not limited to, the central nervous system, including the brain, spinal cord (see, U.S. Pat. Nos. 5,106,627, 5,156,844, and 5,554,148, incorporated by reference), and the aqueous and vitreous humors of the eye (see, WO 97/34586, incorporated by reference).

Methods and apparatus for implantation of capsules into the CNS are described in US 5,487,739. Methods and apparatus for implantation of capsules into the eye are described in US 5,904,144, US 6,299,895, US 6,439,427, and US 20030031700.

In one aspect the invention relates to a biocompatible capsule comprising: a core comprising living packaging cells that secrete a viral vector for infection of a target cell, wherein the viral vector is a vector according to the invention; and an external jacket surrounding said core, said jacket comprising a permeable biocompatible material, said material having a porosity selected to permit passage of retroviral vectors of approximately 100 nm diameter thereacross, permitting release of said viral vector from said capsule.

Preferably, the core additionally comprises a matrix, the packaging cells being immobilized by the matrix. According to one embodiment, the jacket comprises a hydrogel or thermoplastic material.

Examples of suitable cells for packaging cell lines include HEK293, NIH3T3, PG13, and ARPE-19 cells. Preferred cells include PG13 and 3T3 cells.

Packaging cell lines may be encapsulated and administered using the methods and compositions disclosed in US 6,027,721 and WO 97/01357 hereby incorporated by reference in their entirety.

XI Support matrix for NsG33 producing cells

The present invention further comprises culturing NsG33 producing cells in vitro on a support matrix prior to implantation into the mammalian nervous system. The preadhesion of cells to microcarriers prior to implantation is designed to enhance the long-term viability of the transplanted cells and provide long term functional benefit.

To increase the long term viability of the transplanted cells, i.e., transplanted NsG33 secreting cells, the cells to be transplanted can be attached in vitro to a support matrix prior to transplantation. Materials of which the support matrix can be comprised include those materials to which cells adhere following in vitro incubation, and on which cells can grow, and which can be implanted into the mammalian body without producing a toxic reaction, or an inflammatory reaction which would destroy the implanted cells or otherwise interfere with their biological or therapeutic activity. Such materials may be synthetic or natural chemical substances, or substances having a biological origin.

The matrix materials include, but are not limited to, glass and other silicon oxides, polystyrene, polypropylene, polyethylene, polyvinylidene fluoride, polyurethane, polyalginate, polysulphone, polyvinyl alcohol, acrylonitrile polymers, polyacrylamide, polycarbonate, polypentent, nylon, amylases, natural and modified gelatin and natural and codified collagen, natural and modified polysaccharides, including dextrans and celluloses (e.g., nitrocellulose), agar, and magnetite. Either resorbable or non-resorbable materials may be used. Also intended are extracellular matrix materials, which are well-known in the art. Extracellular matrix materials may be obtained commercially or prepared by growing cells which secrete such a matrix, removing the secreting cells, and allowing the cells which are to be transplanted to interact with and adhere to the matrix. The matrix material on which the cells to be implanted grow, or with which the cells are mixed, may be an indigenous product of RPE cells. Thus, for example, the matrix material may be extracellular matrix or basement membrane material, which is produced and secreted by RPE cells to be implanted.

To improve cell adhesion, survival and function, the solid matrix may optionally be coated on its external surface with factors known in the art to promote cell adhesion, growth or survival. Such factors include cell adhesion molecules, extracellular matrix, such as, for example, fibronectin, laminin, collagen, elastin, glycosaminoglycans, or proteoglycans or growth factors.

Alternatively, if the solid matrix to which the implanted cells are attached is constructed of porous material, the growth- or survival promoting factor or factors may be incorporated into the matrix material, from which they would be slowly released after implantation in vivo.

When attached to the support according to the present invention, the cells used for transplantation are generally on the "outer surface" of the support. The support may be solid or porous. However, even in a porous support, the cells are in direct contact with the external milieu without an intervening membrane or other barrier. Thus, according to the present invention, the cells are considered to be on the "outer surface" of the support even though the surface to which they adhere may be in the form of internal folds or convolutions of the porous support material which are not at the exterior of the particle or bead itself.

The configuration of the support is preferably spherical, as in a bead, but may be cylindrical, elliptical, a flat sheet or strip, a needle or pin shape, and the like. A preferred form of support matrix is a glass bead. Another preferred bead is a polystyrene bead.

Bead sizes may range from about 10 µm to 1 mm in diameter, preferably from about 90 µm to about 150 µm. For a description of various microcarrier beads, see, for example, Isher Biotech Source 87-88, Fisher Scientific Co., 1987, pp. 72-75; Sigma Cell Culture Catalog, Sigma Chemical Co., St, Louis, 1991, pp. 162-163; Ventrex Product Catalog, Ventrex Laboratories, 1989; these references are hereby incorporated by reference. The upper limit of the bead's size may be dictated by the bead's stimulation of undesired host reactions, which may interfere with the function of the transplanted cells or cause damage to the surrounding tissue. The upper limit of the bead's size may also be dictated by the method of administration. Such limitations are readily determinable by one of skill in the art.

XII. Host cells

In one aspect the invention relates to isolated host cells genetically modified with the vector according to the invention.

According to one embodiment, the host cells are prokaryotic cells such as E. coli which are capable producing recombinant protein in high quantities and which can easily be scaled up to industrial scale. The use of prokaryotic producer cells may require refolding and glycosylation of the NsG33 in order to obtain a biologically active protein. In another embodiment, the host cells are eukaryotic producer cells from non-mammals, including but not limited to known producer cells such as yeast (Saccharomyces cerevisiae), filamentous fungi such as aspergillus, and insect cells, such as Sf9

According to another embodiment, the cells preferably are mammalian host cells because these are capable of secreting and processing the encoded NsG33 correctly. Preferred species include the group consisting of human, feline, porcine, simian, canina, murine, rat, rabbit, mouse, and hamster.

Examples of primary cultures and cell lines that are good candidates for transduction or transfection with the vectors of the present invention include the group consisting of CHO, CHO-K1, HEI193T, HEK293, COS, PC12, HiB5, RN33b, neuronal cells, foetal cells, ARPE-19, C2C12, HeLa, HepG2, striatal cells, neurons, astrocytes, and interneurons. Preferred cell lines for mammalian recombinant production include CHO, CHO-1, HEI193T, HEK293, COS, PC12, HiB5, RN33b, and BHK cells.

For ex vivo gene therapy, the preferred group of cells include neuronal cells, neuronal precursor cells, neuronal progenitor cells, stem cells and foetal cells.

The invention also relates to cells suitable for biodelivery of NsG33 via naked or encapsulated cells, which are genetically modified to overexpress NsG33, and which can be transplanted to the patient to deliver bioactive NsG33 polypeptide locally. Such cells may broadly be referred to as therapeutic cells.

In a preferred embodiment of the invention, a therapeutic cell line has not been immortalised with the insertion of a heterologous immortalisation gene. As the invention relates to cells which are particularly suited for cell transplantation, whether as naked cells or – preferably as encapsulated cells, such immortalised cell lines are less preferred as there is an inherent risk that they start proliferating in an uncontrolled manner inside the human body and potentially form tumours.

Preferably, the cell line is a contact inhibited cell line. By a contact inhibited cell line is intended a cell line which when grown in 2-D cultures grow to confluency and then substantially stop dividing. This does not exclude the possibility that a limited number of cells escape the 2D layer. Contact inhibited cells may also be grown in 3D, e.g. inside a capsule. Also inside the capsules, the cells grow to confluency and then significantly slow down proliferation rate or completely stop dividing. A particularly preferred type of cells include epithelial cells which are by their nature contact-inhibited and which form stable monolayers in culture.

Even more preferred are retinal pigment epithelial cells (RPE cells). The source of RPE cells is by primary cell isolation from the mammalian retina. Protocols for harvesting RPE cells are well-defined (Li and Turner, 1988, Exp. Eye Res. 47:911-917; Lopez et al., 1989, Invest. Ophthalmol. Vis. Sci. 30:586-588) and considered a routine methodology. In most of the published reports of RPE cell cotransplantation, cells are derived from the rat (Li and Turner, 1988; Lopez et al., 1989). According to the present invention RPE cells are derived from humans. In addition to isolated primary RPE cells, cultured human RPE cell lines may be used in the practice of the invention.

For encapsulation, the cells need to be able to survive and maintain a functional NsG33 secretion at the low oxygen tension levels of the CNS. Preferably the cell line of the invention is capable of surviving at an oxygen tension below 5%, more preferably below 2%, more preferably below 1%. 1% oxygen tension corresponds approximately to the oxygen level in the brain.

To be a platform cell line for an encapsulated cell based delivery system, the cell line should have as many of the following characteristics as possible: (1) The cells should be hardy under stringent conditions (the encapsulated cells should be functional in the vascular and avascular tissue cavities such as in the central nervous system intraparenchymally or within the ventricular or intrathecal fluid spaces or the eye, especially in the intra-ocular environment). (2) The cells should be able to be genetically modified to express NsG33. (3) The cells should have a relatively long life span (the cells should produce sufficient progenies to be banked, characterised, engineered, safety tested and clinical lot manufactured). (4) The cells must be of human origin (which increases compatibility between the encapsulated cells and the host). (5) The cells should exhibit greater than 80% viability for a period of more than one month in vivo in device (which ensures long-term delivery). (6) The encapsulated cells should deliver an efficacious quantity of NsG33 (which ensures effectiveness of the treatment). (7) when encapsulated the cells should not cause a significant host immune reaction (which ensures the longevity of the graft). (8) The cells should be non-tumourigenic (to provide added safety to the host, in case of device leakage).

For encapsulation the preferred cells include retinal pigmented epithelial cells, including ARPE-19 cells; human immortalised fibroblasts; and human immortalised astrocytes.

The ARPE-19 cell line is a superior platform cell line for encapsulated cell based delivery technology and is also useful for unencapsulated cell based delivery technology. The ARPE-19

cell line is hardy (i.e., the cell line is viable under stringent conditions, such as implantation in the central nervous system or the intra-ocular environment). ARPE-19 cells can be genetically modified to secrete a substance of therapeutic interest. ARPE-19 cells have a relatively long life span. ARPE-19 cells are of human origin. Furthermore, encapsulated ARPE-19 cells have good in vivo device viability. ARPE-19 cells can deliver an efficacious quantity of growth factor. ARPE-19 cells elicit a negligible host immune reaction. Moreover, ARPE-19 cells are non-tumorigenic. Methods for culture and encapsulation of ARPE-19 cells are described in US 6,361,771.

In another embodiment the therapeutic cell line is selected from the group consisting of: human fibroblast cell lines, human astrocyte cell lines, human mesencephalic cell line, and human endothelial cell line, preferably immortalised with TERT, SV40T or vmyc.

The method for generating an immortalised human astrocyte cell lines has previously been described (Price TN, Burke JF, Mayne LV. A novel human astrocyte cell line (A735) with astrocyte-specific neurotransmitter function. In Vitro Cell Dev Biol Anim. 1999 May;35(5):279-88.). This protocol may be used to generate astrocyte cell lines.

The following three modifications of that protocol are preferably made to generate additional human astrocyte cell lines.

Human foetal brain tissue dissected from 5-12 weeks old foetuses may be used instead of 12-16 weeks old tissue.

The immortalisation gene *v-myc*, or TERT (telomerase) may be used instead of the *SV40 T antigen*.

Retroviral gene transfer may be used instead of transfection with plasmids by the calcium phosphate precipitation technique.

XIII Recombinant production and purification of NsG33 polypeptides of the invention

The NsG33 polypeptides of the invention may be produced using state of the art prokaryotic or eukaryotic expression systems. Examplary methods are described in WO 93/22437 (Innogenetics), which is hereby incorporated by reference. Due to the structural similarity between NsG33 polypeptides and the polypeptides described in WO 93/22437 it is contemplated that NsG33 polypeptides can be produced using the production methods described in this publication. The protocols described in WO 93/22437 describe purification of a

protein having a predicted molecular weight of 29 kDa. In the case of expression of NsG33 fragments, which may be considerably shorter due to possible propeptide cleavage, the protocols should be modified to take the difference in molecular weight into consideration.

These examples include expression in E. coli (Example 5 of WO 93/22437), expression in COS1 cells (Example 6 of WO 93/22437), expression in a baculovirus expression system (Example 7 of WO 93/22437), expression in a vaccinia virus system (Example 8 of WO 93/22437). Each of the referenced expression systems resulted in the expression of significant amounts of the polypeptides described in WO 93/22437.

Purification of NsG33 proteins may be performed using the purification method described in WO 93/22437. Briefly, conditioned medium of COS1 cells transfected with the cDNA of the invention is collected after 48 h and filtered over a 0.22 µm filter to remove cell debris. A typical purification starts from 600 to 1000 ml of COS1 transfection medium. To this $MgCl_2$ and dextrane-sulphate 500.000 (Pharmacia, Uppsala, Sweden) is added to a final concentration of 60 mM and 0.02%, respectively. After 1 h incubation at 4°C the precipitate is pelleted by centrifugation (12.000 g, 30 min., 4°C). The supernatant fraction, containing the NsG33 is dialysed against 50 mM Hepes pH 7.0, 4 mM EDTA, adjusted to pH 8.0 and loaded at a flowrate of 0.5 ml/minute on a 4 ml Phenylboronate agarose (PBA 30, Amicon, MA, USA) column equilibrated in 50 mM Hepes pH 8.5. The NsG33 is eluted from the matrix by 100 mM Sorbitol.

The Sorbitol eluated peak is then passed at a flowrate of 0.5ml/minute over a 1 ml FPLC Mono Q anion exchange column (Pharmacia) equilibrated in Hepes pH 8.5 and eluted with a linear salt gradient of 0 to 1 M NaCl at a flowrate of 1 ml/minute.

The eluate is concentrated about 40 fold by Centricon 10.000 (Amicon) and loaded batchwise (3 times 0.25 ml) on a SMART Superdex 75 gelfiltration column (Pharmacia) equilibrated against PBS. This protocol may result in elution of protein of high purity.

Other state of the art protein purification protocols may also be used to provide enough pure protein to perform the in vitro and in vivo assays described in the examples.

EXAMPLES

Example 1, NsG 33 sequences

SEQ ID NO 1, human NsG33 genomic sequence with 100 extra basepairs added in the ends of 5'and 3'.

SEQ ID NO 2, human NsG33 cDNA

SEQ ID NO 3, human NsG33 full length amino acid sequence

SEQ ID NO 4, human NsG33 protein without signal peptide

SEQ ID NO 5, human NsG33 C-terminal polypeptide

SEQ ID NO 6, mouse NsG33 genomic sequence with 100 extra basepairs added in the ends of 5' and 3'.

SEQ ID NO 7, mouse NsG33 cDNA

SEQ ID NO 8, mouse NsG33 partial amino acid sequence

SEQ ID NO 9, mouse NsG33 protein without signal peptide

SEQ ID NO 10, mouse NsG33 C-terminal polypeptide

SEQ ID NO 11, rat NsG33 genomic sequence with 100 extra basepairs added in the ends of 5' and 3'.

SEQ ID NO 12, rat NsG33 cDNA

SEQ ID NO 13, rat NsG33 full length amino acid sequence

SEQ ID NO 14, rat NsG33, protein without signal peptide

SEQ ID NO 15, rat NsG33, C-terminal polypeptide

SEQ ID No 16, nucleotide sequence encoding human C-terminal peptide NsG33

SEQ ID No 17, nucleotide sequence encoding mouse C-terminal peptide NsG33

SEQ ID No 18, nucleotide sequence encoding rat C-terminal peptide NsG33

SEQ ID No 19, human N-terminal peptide

SEQ ID No 20, mouse N-terminal peptide

SEQ ID No 21, rat N-terminal peptide

SEQ ID No 22, human N-terminal peptide

SEQ ID No 23, mouse N-terminal peptide

SEQ ID No 24, rat N-terminal peptide

In the sequence listing, introns are marked in lowercase and exons in UPPERCASE. In the polypeptide sequences, signal peptides are marked in **bold**.

Human NsG33 genomic nucleotide sequence (SEQ ID NO 1)

```
actggccgac acgccgcagg ccccgccccc ttcccgaccc gctccaaggc  0050
ggccccggcg ctggggctgc gcggcaggcg gagcggccgc gggcttgggg  0100
GCTTCGCCGG GGCCGGGCGG CCGGCGCCCC CGGCTGCTCC CGCCGCCGCC  0150
CGGACCCGCG CCCCGCCGGG GCAGCGGTGG TGAGAGCCCC GACTCCCCGG  0200
ACGCCGCCCG CCGTGCCATG GGGTTCCCGG CCGCGGCGCT GCTCTGCGCG  0250
CTGTGCTGCG GCCTCCTGGC CCCGGCTGCC CGCGCCGGCT ACTCCGAGGA  0300
```

GCGCTGCAGC TGGAGGGGCA Ggtacggtcc gggggggctgt ccccgcactt 0350
aggacggggt gcgctgcggc taggaccccc caggcgcccc tcggagcgcg 0400
cagagcgctg ggccggtttc cccatccgcg aggcggcctc gggagggagc 0450
gggggctgcg ccgggcgggg acccgccccc gtctcagcgc cccgtcccgt 0500
cctgtcccca gCGGCCTCAC CCAGGAGCCC GGCAGCGTGG GGCAGCTGGC 0550
CCTGGCCTGT GCGGAGGGCG CGGTTGAGTG GCTGTACCCG GCTGGGGCGC 0600
TGCGCCTGAC CCTGGGCGGC CCCGATCCCA GAGCGCGGCC CGGCATCGCC 0650
TGTCTGCGGC CGGTGCGGCC CTTCGCGGGC GCCCAGGTCT TCGCGGAGCG 0700
CGCAGGGGGC GCCCTGGAGC TGCTGCTGGC CGAGGGCCCG GGCCCGGCAG 0750
GGGGCCGCTG CGTGCGCTGG GGTCCCCGCG AGCGCCGGGC CCTCTTCCTG 0800
CAGGCCACGC CGCACCAGGA CATCAGCCGC CGCGTGGCCG CCTTCCGCTT 0850
TGAGCTGCGC GAGGACGGGC GCCCCGAGCT GCCCCCGCAG GCCCACGGTC 0900
TCGGCGTAGA CGgtgagtgg cggtctggtt gggacagggt gggagtcccg 0950
aagtcttacc ctgcctgggc ttggcgggaa tgtgccttgt cggccccact 1000
gcagaaggaa aaagtgagct acaagggttg gatgggcttg tcaggccaca 1050
cagcctggga ctgctgggga gggatggcct ccccgccctc ccttcccgat 1100
tcatctctgg aaagagctgg caggggcaga gtggagggaa ggggaggccg 1150
ggcccagcaa tcctgggcct ctggtccctg aacggttggg ggaagagatg 1200
gtggggacag aatcgaagcc tccggccaaa gctgtccggg gctccctggc 1250
ccagcggtga cctctctccc ctcccccagc ccaaccaaca aaagtccagt 1300
gtgcagcccg gtcaccatgg agacgccgct cgcctccctg cagggcacca 1350
ggcccagctc ttgcttggct ctcctggagc ttggcgcctg accctgaaag 1400
ggatgggctc tcgctattct gccccctggc cctgggccag ggaccccaga 1450
ccacccttcc tctgccccca cttcctatca ccctagctgg gctgctgctc 1500
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CGCATGCACC AGCGACTTCG gtgagtgtcc ccgccatggg gggagcctgg 1950
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ATTCACGGGA TCATCCATGG GGTCACCCAT GACGTGGAGC TGCAGGAGTC 2050
TGTCATCACT GTGGTGGCCG CCCGTGTCCT CCGCCAGACA CCGCCGCTGT 2100
TCCAGGCGGG GCGATCCGGG GACCAGGGGC TGACCTCCAT TCGTACCCCA 2150
CTGCGCTGTG GCGTCCACCC GGGCCCAGGC ACCTTCCTCT TCATGGGCTG 2200
GAGCCGCTTT GGGGAGGCCC GGCTGGGCTG TGCCCCACGA TTCCAGGAGT 2250
TCCGCCGTGC CTACGAGGCT GCCCGTGCTG CCCACCTCCA CCCCTGCGAG 2300
GTGGCGCTGC ACTGAGGGGC TGGGTGCTGG GGAGGGGCTG GTAGGAGGGA 2350
GGGTGGGCCC ACTGCTTTGG AGGTGATGGG ACTATCAATA AGAACTCTGT 2400
TCACGCAAgc tgctgtggac ctggtctcct gtgtccagcc cagccttggg 2450
cctgcctcgc agctgtgagg atggctccaa ttcctgcctc ctggcgggag 2500
actgaggc

Human NsG33 (1109 bp; CDS=118-999) (SEQ ID NO 2)

>gi|34147349|ref|NM_024042.2| Homo sapiens hypothetical protein MGC2601 (MGC2601), mRNA

GCTTCGCCGGGGCCGGGCGGCCGGCGCCCCCGGCTGCTCCCGCCGCCGCCCGGACCCGCGCCCCGCCGGG
GCAGCGGTGGTGAGAGCCCCGACTCCCCGGACGCCGCCCGCCGTGCCATGGGGTTCCCGGCCGCGGCGCT
GCTCTGCGCGCTGTGCTGCGGCCTCCTGGCCCCGGCTGCCCGCGCCGGCTACTCCGAGGAGCGCTGCAGC
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CGGTTGAGTGGCTGTACCCGGCTGGGGCGCTGCGCCTGACCCTGGGCGGCCCCGATCCCAGAGCGCGGCC
CGGCATCGCCTGTCTGCGGCCGGTGCGGCCCTTCGCGGGCGCCCAGGTCTTCGCGGAGCGCGCAGGGGGC
GCCCTGGAGCTGCTGCTGGCCGAGGGCCCGGGCCCGGCAGGGGGCCGCTGCGTGCGCTGGGGTCCCCGCG
AGCGCCGGGCCCTCTTCCTGCAGGCCACGCCGCACCAGGACATCAGCCGCCGCGTGGCCGCCTTCCGCTT
TGAGCTGCGCGAGGACGGGCGCCCCGAGCTGCCCCCGCAGGCCCACGGTCTCGGCGTAGACGGTGCCTGC
AGGCCCTGCAGCGACGCTGAGCTGCTCCTGGCCGCATGCACCAGCGACTTCGTAATTCACGGGATCATCC
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GACACCGCCGCTGTTCCAGGCGGGGCGATCCGGGGACCAGGGGCTGACCTCCATTCGTACCCCACTGCGC
TGTGGCGTCCACCCGGGCCCAGGCACCTTCCTCTTCATGGGCTGGAGCCGCTTTGGGGAGGCCCGGCTGG
GCTGTGCCCCACGATTCCAGGAGTTCCGCCGTGCCTACGAGGCTGCCCGTGCTGCCCACCTCCACCCCTG
CGAGGTGGCGCTGCACTGAGGGGCTGGGTGCTGGGGAGGGGCTGGTAGGAGGGAGGGTGGGCCCACTGCT
TTGGAGGTGATGGGACTATCAATAAGAACTCTGTTCACGCAAAAAAAAAAAAAAAAAAA

Nucleotide sequence encoding human NsG33 C-terminal polypeptide (SEQ ID NO 16)

GCCCTCTTCCTGCAGGCCACGCCGCACCAGGACATCAGCCGCCGCGTGGCCGCCTTCCGCTTTGAGCTGC
GCGAGGACGGGCGCCCCGAGCTGCCCCCGCAGGCCCACGGTCTCGGCGTAGACGGTGCCTGCAGGCCCTG
CAGCGACGCTGAGCTGCTCCTGGCCGCATGCACCAGCGACTTCGTAATTCACGGGATCATCCATGGGGTC
ACCCATGACGTGGAGCTGCAGGAGTCTGTCATCACTGTGGTGGCCGCCCGTGTCCTCCGCCAGACACCGC
CGCTGTTCCAGGCGGGGCGATCCGGGGACCAGGGGCTGACCTCCATTCGTACCCCACTGCGCTGTGGCGT
CCACCCGGGCCCAGGCACCTTCCTCTTCATGGGCTGGAGCCGCTTTGGGGAGGCCCGGCTGGGCTGTGCC
CCACGATTCCAGGAGTTCCGCCGTGCCTACGAGGCTGCCCGTGCTGCCCACCTCCACCCCTGCGAGGTGG
CGCTGCAC

Human NsG33 full length amino acid sequence (SEQ ID NO 3)

>IPI00031531.1 REFSEQ_NP:NP_076947 TREMBL:Q9UJH9 ENSEMBL:ENSP00000219542 Tax_Id=9606 C380A1.2.1 (Novel protein)

MGFPAAALLC ALCCGLLAPA ARAGYSEERC SWRGSGLTQE PGSVGQLALA CAEGAVEWLY
PAGALRLTLG GPDPRARPGI ACLRPVRPFA GAQVFAERAG GALELLLAEG PGPAGGRCVR
WGPRERRALF LQATPHQDIS RRVAAFRFEL REDGRPELPP QAHGLGVDGA CRPCSDAELL
LAACTSDFVI HGIIHGVTHD VELQESVITV VAARVLRQTP PLFQAGRSGD QGLTSIRTPL
RCGVHPGPGT FLFMGWSRFG EARLGCAPRF QEFRRAYEAA RAAHLHPCEV ALH

Human NsG33, protein without signal peptide (SEQ ID NO 4)

GYSEERCSWR GSGLTQEPGS VGQLALACAE GAVEWLYPAG ALRLTLGGPD PRARPGIACL
RPVRPFAGAQ VFAERAGGAL ELLLAEGPGP AGGRCVRWGP RERRALFLQA TPHQDISRRV
AAFRFELRED GRPELPPQAH GLGVDGACRP CSDAELLLAA CTSDFVIHGI IHGVTHDVEL
QESVITVVAA RVLRQTPPLF QAGRSGDQGL TSIRTPLRCG VHPGPGTFLF MGWSRFGEAR
LGCAPRFQEF RRAYEAARAA HLHPCEVALH

Human NsG33, C-terminal polypeptide (SEQ ID NO 5)

ALFLQATPHQ DISRRVAAFR FELREDGRPE LPPQAHGLGV DGACRPCSDA ELLLAACTSD
FVIHGIIHGV THDVELQESV ITVVAARVLR QTPPLFQAGR SGDQGLTSIR TPLRCGVHPG
PGTFLFMGWS RFGEARLGCA PRFQEFRRAY EAARAAHLHP CEVALH

Human N-terminal peptide (SEQ ID No 19)

GYSEERCSWR GSGLTQEPGS VGQLALACAE GAVEWLYPAG ALRLTLGGPD PRARPGIACL
RPVRPFAGAQ VFAERAGGAL ELLLAEGPGP AGGRCVRWGP RERR

Human N-terminal peptide (SEQ ID No 22)

GYSEERCSWR GSGLTQEPGS VGQLALACAE GAVEWLYPAG ALRLTLGGPD PRARPGIACL
RPVRPFAGAQ VFAERAGGAL ELLLAEGPGP AGGRCVR

Mouse NsG33 genomic nucleotide sequence (SEQ ID NO 6)

Genomic chr17 (reverse strand):

nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn 0050

```
nnnnnnnnnn nnnnnnnnnn nnnnnnnncc cctaaccatg ctggtagcca  0100
CGCTTCTTTG CGCGCTCTGT TGCGGCCTCC TGGCCGCGTC CGCTCACGCT  0150
GGCTACTCGG AAGACCGCTG CAGCTGGAGG GGCAGgtacc aggagggact  0200
gcggggaggg ttgtgggttt atttatttat ttattttatt ttatttactt  0250
cttgggttgg agggttccct cccacttgga actgaggaaa cgcagacttc  0300
aatgtcctgt tacacagagt agaagcagat gttggtagcc gcgggaaaag  0350
ggatgagcgg gctagggaac gagggtcacc cacctgagaa ccaccgtcct  0400
gtccccagCG GTTTGACCCA GGAGCCTGGC AGCGTGGGGC AGCTGACCCT  0450
GGACTGTACT GAGGGCGCTA TCGAGTGGCT GTACCCAGCT GGGGCGCTGC  0500
GCCTGACCCT GGGCGGCCCC GATCCGGGCA CACGGCCCAG CATCGTCTGT  0550
CTGCGCCCAG AGCGGCCCTT CGCTGGTGCC CAGGTCTTCG CTGAACGTAT  0600
GACCGGCAAT CTAGAGTTGC TACTGGCCGA GGGCCCGGAC CTGGCTGGGG  0650
GCCGCTGCAT GCGCTGGGGT CCCCGCGAGC GCCGAGCCCT TTTCCTGCAG  0700
GCCACACCAC ACCGCGACAT CAGCCGCAGA GTTGCTGCCT TCCGTTTTGA  0750
ACTGCACGAG GACCAACGTG CAGAAATGTC TCCCCAGGCT CAAGGTCTTG  0800
GTGTGGATGg tgagtgatta tgagactggc tgggtgtcag aaattggccc  0850
tccacactga cctgatggga ctgggccttg ccaccccatt gcatggagag  0900
tccttctgta gcttgacaga ggccactccg gtggagagca tagtggcttc  0950
caggtcgtaa ggaggtgagt tggaagtgcc cccgcctttc tctcctcctc  1000
ctcttaaaag attcggttta ggaaaagagc aggagggggc aaatgcccga  1050
gaggccagcc ctgggtctct ggtttctgaa ggattggggg aagggttaag  1100
ctgaggcaga atcaaagcct atggccaagg ctgtccaggg ctccctggcc  1150
tggtggtgac ctccttcccc tccccccaag cccagccaac aaaagtccag  1200
tgtgcctctt cgtcaccatg gagactgcct gccctgcctc cctgcagggc  1250
accaggccca gtgctttgct cttctggaac ttgtagcctg accctgcagg  1300
gaatgaatgg ctctctgact gttctgccct agctagagac ccccccgaac  1350
tggagtccac tagaatatcc ctagctagag ctgggaggtc acagaacgtt  1400
tcccagtgtt agtctgagtt tatgagatgg taccaagcct gtgtatgagg  1450
cactgaggtg cccatcagta ggcatgtacc tgcagggtgt cttcaggcta  1500
taggatgctg ggagaagggt ttagtctctt gctcctgtac cttttcctct  1550
tgggaggagc tgtgggctcg tgctgagaga tcacaggcct ggctgatgac  1600
ctgccttgca tgctagGTGC CTGCAGGCCC TGCAGTGATG CCGAGCTCCT  1650
CCTGGCTGCA TGCACCAGTG ATTTTGgtga gtgtttctgt tgcgggagag  1700
cttagggtct gcctcacatt cccacgtgcc caccactggc caccatgtct  1750
cctcgtagTG ATCCACGGGA CCATCCATGG GGTCGCCCAT GACACAGAGC  1800
TGCAAGAATC AGTCATCACT GTGGTGGTTG CTCGTGTCAT CCGCCAGACA  1850
CTGCCACTGT TCAAGGAAGG GAGCTCGGAG GGCCAAGGCC GGGCCTCCAT  1900
TCGTACCTTG CTGCGCTGTG GTGTGCGTCC TGGCCCAGGC TCCTTCCTCT  1950
TCATGGGCTG GAGCCGATTT GGCGAAGCTT GGCTGGGCTG TGCTCCCCGC  2000
TTCCAAGAGT TCAGCCGTGT CTATTCAGCT GCTCTCACGA CCCATCTCAA  2050
CCCATGTGAG ATGGCACTGG ACTGAGAGAC CTGGGAGCAA GCCCTGGATG  2100
GACCTTCTTC TGGAGATGGG GTGTTGGGGA GGGTGATGGG AGGGTGGGTG  2150
AGAAGGGTGT GGCTCGGATG GCATCCTGGT ACCCACAGTG AGCTGGTAGA  2200
ATACTAAGTA ATCTGGACCA TAccagccac tgtagtcatg gtcttctgtg  2250
gcaggcagca tacccagctc tgtgcctgcc tcactttgtc tactctccag  2300
tctgctgccc ttctaaccct tc
```

Mouse NsG33 cDNA (1048 bp; CDS= <2 - 886) (SEQ ID NO 7)

```
CCACGCGTCCGCCCACGCGTCCGCGCTTCTTTGCGCGCTCTGTTGCGGCCTCCTGGCCGCGTCCGCTCAC
GCTGGCTACTCGGAAGACCGCTGCAGCTGGAGGGGCAGCGGTTTGACCCAGGAGCCTGGCAGCGTGGGGC
AGCTGACCCTGGACTGTACTGAGGGCGCTATCGAGTGGCTGTACCCAGCTGGGGCGCTGCGCCTGACCCT
```

GGGCGGCCCCGATCCGGGCACACGGCCCAGCATCGTCTGTCTGCGCCCAGAGCGGCCCTTCGCTGGTGCC
CAGGTCTTCGCTGAACGTATGACCGGCAATCTAGAGTTGCTACTGGCCGAGGGCCCGGACCTGGCTGGGG
GCCGCTGCATGCGCTGGGGTCCCCGCGAGCGCCGAGCCCTTTTCCTGCAGGCCACACCACACCGCGACAT
CAGCCGCAGAGTTGCTGCCTTCCGTTTTGAACTGCACGAGGACCAACGTGCAGAAATGTCTCCCCAGGCT
CAAGGTCTTGGTGTGGATGGTGCCTGCAGGCCCTGCAGTGATGCCGAGCTCCTCCTGGCTGCATGCACCA
GTGATTTTGTGATCCACGGGACCATCCATGGGGTCGCCCATGACACAGAGCTGCAAGAATCAGTCATCAC
TGTGGTGGTTGCTCGTGTCATCCGCCAGACACTGCCACTGTTCAAGGAAGGGAGCTCGGAGGGCCAAGGC
CGGGCCTCCATTCGTACCTTGCTGCGCTGTGGTGTGCGTCCTGGCCCAGGCTCCTTCCTCTTCATGGGCT
GGAGCCGATTTGGCGAAGCTTGGCTGGGCTGTGCTCCCCGCTTCCAAGAGTTCAGCCGTGTCTATTCAGC
TGCTCTCACGACCCATCTCAACCCATGTGAGATGGCACTGGACTGAGAGACCTGGGAGCAAGCCCTGGAT
GGACCTTCTTCTGGAGATGGGGTGTTGGGGAGGGTGATGGGAGGGTGGGTGAGAAGGGTGTGGCTCGGAT
GGCATCCTGGTACCCACAGTGAGCTGGTAGAATACTAAGTAATCTGGACCATAAAAAAAAAAAAAAAA

Nucleotide sequence encoding ouse C-terminal polypeptide NsG33 (SEQ ID No 17)

GCCCTTTTCCTGCAGGCCACACCACACCGCGACATCAGCCGCAGAGTTGCTGCCTTCCGTTTTGAACTGC
ACGAGGACCAACGTGCAGAAATGTCTCCCCAGGCTCAAGGTCTTGGTGTGGATGGTGCCTGCAGGCCCTG
CAGTGATGCCGAGCTCCTCCTGGCTGCATGCACCAGTGATTTTGTGATCCACGGGACCATCCATGGGGTC
GCCCATGACACAGAGCTGCAAGAATCAGTCATCACTGTGGTGGTTGCTCGTGTCATCCGCCAGACACTGC
CACTGTTCAAGGAAGGGAGCTCGGAGGGCCAAGGCCGGGCCTCCATTCGTACCTTGCTGCGCTGTGGTGT
GCGTCCTGGCCCAGGCTCCTTCCTCTTCATGGGCTGGAGCCGATTTGGCGAAGCTTGGCTGGGCTGTGCT
CCCCGCTTCCAAGAGTTCAGCCGTGTCTATTCAGCTGCTCTCACGACCCATCTCAACCCATGTGAGATGG
CACTGGAC

Mouse NsG33 partial NsG33 (SEQ ID NO 8), i.e. missing N-ternimal

>gi|23274274|gb|AAH37181.1| 1810034B16Rik protein [Mus musculus]
HASAHASALL CALCCGLLAA SAHAGYSEDR CSWRGSGLTQ EPGSVGQLTL DCTEGAIEWL YPAGALRLTL
GGPDPGTRPS IVCLRPERPF AGAQVFAERM TGNLELLLAE GPDLAGGRCM RWGPRERRAL FLQATPHRDI
SRRVAAFRFE LHEDQRAEMS PQAQGLGVDG ACRPCSDAEL LLAACTSDFV IHGTIHGVAH DTELQESVIT
VVVARVIRQT LPLFKEGSSE GQGRASIRTL LRCGVRPGPG SFLFMGWSRF GEAWLGCAPR FQEFSRVYSA
ALTTHLNPCE MALD

Mouse NsG33 protein without signal peptide (SEQ ID NO 9)

GYSEDRCSWR GSGLTQEPGS VGQLTLDCTE GAIEWLYPAG ALRLTLGGPD PGTRPSIVCL RPERPFAGAQ
VFAERMTGNL ELLLAEGPDL AGGRCMRWGP RERRALFLQA TPHRDISRRV AAFRFELHED QRAEMSPQAQ
GLGVDGACRP CSDAELLLAA CTSDFVIHGT IHGVAHDTEL QESVITVVVA RVIRQTLPLF KEGSSEGQGR
ASIRTLLRCG VRPGPGSFLF MGWSRFGEAW LGCAPRFQEF SRVYSAALTT HLNPCEMALD

Mouse NsG33, C-terminal polypeptide (SEQ ID No 10)

ALFLQATPHR DISRRVAAFR FELHEDQRAE MSPQAQGLGV DGACRPCSDA ELLLAACTSD FVIHGTIHGV
AHDTELQESV ITVVVARVIR QTLPLFKEGS SEGQGRASIR TLLRCGVRPG PGSFLFMGWS RFGEAWLGCA
PRFQEFSRVY SAALTTHLNP CEMALD

Mouse NsG33, N-terminal polypeptide (SEQ ID No 20)

GYSEDRCSWR GSGLTQEPGS VGQLTLDCTE GAIEWLYPAG ALRLTLGGPD PGTRPSIVCL RPERPFAGAQ
VFAERMTGNL ELLLAEGPDL AGGRCMRWGP RERR

Mouse NsG33, N-terminal polypeptide (SEQ ID No 23)

GYSEDRCSWR GSGLTQEPGS VGQLTLDCTE GAIEWLYPAG ALRLTLGGPD PGTRPSIVCL RPERPFAGAQ
VFAERMTGNL ELLLAEGPDL AGGRCMR

Rat NsG33 genomic sequence with 100 extra basepairs added in the ends of 5'and 3' (SEQ ID NO 11). Genomic chr10 (reverse strand):

tccccggttg tggggannnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn 15064142
nnnnnnnnnn nnnnnnggca gcagcccgag ccccggcgcg tcccctaacc 15064092
ATGCTGGTAG CGGCGCTTCT CTGCGCGCTG TGCTGCGGCC TCTTGGCTGC 15064042
GTCCGCTCGA GCTGGCTACT CCGAGGACCG CTGCAGCTGG AGGGGCAGgt 15063992

acccaggaga gattttgggg aggatttttg ttatttgtgt tttaaattga 15063942
aatcttgggt tggagggctc cctcccactt ggaactgagg aagcgcagac 15063892
ctcaatgtcc tgttccagag ggtggacgca ggtgttggtg gccgcgggaa 15063842
aagggttgag cgggctaggg aaatgagggc cacccacctg agaaccaccg 15063792
tcctgtcccc agCGGTTTGA CCCAGGAACC TGGCAGCGTG GGGCAGCTGA 15063742
CCCTGGATTG TACTGAGGGT GCTATCGAGT GGCTGTATCC AGCTGGGGCG 15063692
CTGCGCCTGA CTCTAGGCGG CTCTGATCCG GGCACGCGGC CCAGCATCGT 15063642
CTGTCTGCGC CCAACACGGC CCTTCGCTGG TGCCCAGGTC TTCGCTGAAC 15063592
GGATGGCCGG CAACCTAGAG TTGCTACTGG CCGAGGGCCA AGGCCTGGCT 15063542
GGGGGCCGCT GCATGCGCTG GGGTCCTCGC GAGCGCCGAG CCCTTTTCCT 15063492
GCAGGCCACG CCACACCGGG ACATCAGCCG CAGAGTTGCT GCCTTCCAAT 15063442
TTGAACTGCA CGAGGACCAA CGTGCAGAAA TGTCTCCCCA GGCCCAAGGT 15063392
TTTGGTGTGG ATGgtgagtg actagactgg ctggggcgga gctgggtgtc 15063342
agaaactggc cctctacact ggcctgatcc gaatgggcct tgcctcccca 15063292
ctgcaccgaa agccctgtag cttgacggag gctactctgg tggagaacac 15063242
agtggcttcc aggtcatagg gaggtgagtt gagagttctc cctcctttct 15063192
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ccagagaggc cagccttggg tctctggttt ctgaagggtt gggggggaagg 15063092
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tccagtgtgc ctcttcgtca ccatggagac tgcctgccct gcctcccggc 15062942
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agagtccact agaatatccc tggctggacc tgggaggcag ctctgggagg 15062792
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gaatgtgtcc gaggcactga ggggcccatg agtagtcttc aggcagtgtg 15062692
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gggtctgccc cacattccca cgtgcccacc actggccacc atgtctcttc 15062442
gtagTGATCC ATGGGACCAT CCATGGGGTC GTCCATGACA TGGAGCTGCA 15062392
AGAATCAGTC ATCACTGTGG TGGCCACTCG TGTCATCCGC CAGACACTGC 15062342
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ACCTTGTTGC GCTGTGGTGT GCGTCCTGGC CCAGGCTCCT TCCTCTTCAT 15062242
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AAGAGTTCAG CCGTGTCTAT TCAGCTGCTC TCGCGGCCCA CCTCAACCCA 15062142
TGTGAGGTGG CACTGGACTG AGAGACCTGG GAGCAAGCCC TGGATGGATC 15062092
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CTAAATAACC TGGATCACAC Cagccactgt agacatggtc ttctgtgaca 15061942
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tgctgccctt ctgacccttc t

SEQ ID NO 12, rat NsG33 (1026 bp; CDS=1-876)

>gi|34870570|ref|XM_213261.2| Rattus norvegicus similar to 1810034B16Rik protein (LOC287151), mRNA
ATGCTGGTAGCGGCGCTTCTCTGCGCGCTGTGCTGCGGCCTCTTGGCTGCGTCCGCTCGAGCTGGCTACT
CCGAGGACCGCTGCAGCTGGAGGGGCAGCGGTTTGACCCAGGAACCTGGCAGCGTGGGGCAGCTGACCCT
GGATTGTACTGAGGGTGCTATCGAGTGGCTGTATCCAGCTGGGGCGCTGCGCCTGACTCTAGGCGGCTCT
GATCCGGGCACGCGGCCCAGCATCGTCTGTCTGCGCCCAACACGGCCCTTCGCTGGTGCCCAGGTCTTCG

CTGAACGGATGGCCGGCAACCTAGAGTTGCTACTGGCCGAGGGCCAAGGCCTGGCTGGGGGCCGCTGCAT
GCGCTGGGGTCCTCGCGAGCGCCGAGCCCTTTTCCTGCAGGCCACGCCACACCGGGACATCAGCCGCAGA
GTTGCTGCCTTCCAATTTGAACTGCACGAGGACCAACGTGCAGAAATGTCTCCCCAGGCCCAAGGTTTTG
GTGTGGATGGTGCCTGCAGGCCCTGCAGTGATGCCGAGCTCCTTCTGACTGCATGCACCAGTGACTTTGT
GATCCATGGGACCATCCATGGGGTCGTCCATGACATGGAGCTGCAAGAATCAGTCATCACTGTGGTGGCC
ACTCGTGTCATCCGCCAGACACTGCCACTGTTCCAGGAAGGGAGCTCGGAGGGCCGGGGCCAGGCCTCCG
TTCGTACCTTGTTGCGCTGTGGTGTGCGTCCTGGCCCAGGCTCCTTCCTCTTCATGGGCTGGAGCCGATT
TGGCGAAGCTTGGCTGGGCTGCGCTCCCCGCTTCCAAGAGTTCAGCCGTGTCTATTCAGCTGCTCTCGCG
GCCCACCTCAACCCATGTGAGGTGGCACTGGACTGAGAGACCTGGGAGCAAGCCCTGGATGGATCTTCCT
CTGGGGATGGGGTGTTGGGGAGGGGTGATAGGAGGGTGGGTGGGAAGGGTGTGGCTCAGATGGCATCCTG
GTACCCACAGTGAGGTGGTAGAATACTAAATAACCTGGATCACACC

Rat C-terminal polypeptide NsG33 coding sequence (SEQ ID NO 18)

GCCCTTTTCCTGCAGGCCACGCCACACCGGGACATCAGCCGCAGAGTTGCTGCCTTCCAA
TTTGAACTGCACGAGGACCAACGTGCAGAAATGTCTCCCCAGGCCCAAGGTTTTGGTGTG
GATGGTGCCTGCAGGCCCTGCAGTGATGCCGAGCTCCTTCTGACTGCATGCACCAGTGAC
TTTGTGATCCATGGGACCATCCATGGGGTCGTCCATGACATGGAGCTGCAAGAATCAGTC
ATCACTGTGGTGGCCACTCGTGTCATCCGCCAGACACTGCCACTGTTCCAGGAAGGGAGC
TCGGAGGGCCGGGGCCAGGCCTCCGTTCGTACCTTGTTGCGCTGTGGTGTGCGTCCTGGC
CCAGGCTCCTTCCTCTTCATGGGCTGGAGCCGATTTGGCGAAGCTTGGCTGGGCTGCGCT
CCCCGCTTCCAAGAGTTCAGCCGTGTCTATTCAGCTGCTCTCGCGGCCCACCTCAACCCA
TGTGAGGTGGCACTGGAC

Rat NsG33 full length amino acid sequence (SEQ ID NO 13)

>IPI00369281.1 |REFSEQ_XP:XP_213261|ENSEMBL:ENSRNOP00000026676
MLVAALLCAL CCGLLAASAR AGYSEDRCSW RGSGLTQEPG SVGQLTLDCT EGAIEWLYPA
GALRLTLGGS DPGTRPSIVC LRPTRPFAGA QVFAERMAGN LELLLAEGQG LAGGRCMRWG
PRERRALFLQ ATPHRDISRR VAAFQFELHE DQRAEMSPQA QGFGVDGACR PCSDAELLLT
ACTSDFVIHG TIHGVVHDME LQESVITVVA TRVIRQTLPL FQEGSSEGRG QASVRTLLRC
GVRPGPGSFL FMGWSRFGEA WLGCAPRFQE FSRVYSAALA AHLNPCEVAL D

Rat NsG33, protein without signal peptide (SEQ ID No 14)

ASARAGYSED RCSWRGSGLT QEPGSVGQLT LDCTEGAIEW LYPAGALRLT LGGSDPGTRP
SIVCLRPTRP FAGAQVFAER MAGNLELLLA EGQGLAGGRC MRWGPRERRA LFLQATPHRD
ISRRVAAFQF ELHEDQRAEM SPQAQGFGVD GACRPCSDAE LLLTACTSDF VIHGTIHGVV
HDMELQESVI TVVATRVIRQ TLPLFQEGSS EGRGQASVRT LLRCGVRPGP GSFLFMGWSR
FGEAWLGCAP RFQEFSRVYS AALAAHLNPC EVALD

Rat NsG33, C-terminal polypeptide (SEQ ID No 15)

ALFLQATPHR DISRRVAAFQ FELHEDQRAE MSPQAQGFGV DGACRPCSDA ELLLTACTSD
FVIHGTIHGV VHDMELQESV ITVVATRVIR QTLPLFQEGS SEGRGQASVR TLLRCGVRPG
PGSFLFMGWS RFGEAWLGCA PRFQEFSRVY SAALAAHLNP CEVALD

Rat NsG33, N-terminal polypeptide (SEQ ID No 21)

ASARAGYSED RCSWRGSGLT QEPGSVGQLT LDCTEGAIEW LYPAGALRLT LGGSDPGTRP
SIVCLRPTRP FAGAQVFAER MAGNLELLLA EGQGLAGGRC MRWGPRERR

Rat NsG33, N-terminal polypeptide (SEQ ID No 24)

ASARAGYSED RCSWRGSGLT QEPGSVGQLT LDCTEGAIEW LYPAGALRLT LGGSDPGTRP
SIVCLRPTRP FAGAQVFAER MAGNLELLLA EGQGLAGGRC MR

Example 2, Bioinformatics analysis

General description:

Human NsG33 is a secreted growth factor protein expressed espressed as a 293 amino acid precursor at high levels in the central nervous system and subregions thereof, in the peripheral

nervous system, in the retina, and in the human developing Mesenphalon. The mouse (SEQ ID No 8) and rat (SEQ ID No 13) homologues have full lengths of 294 and 291 amino acids and the % identities are 80.3 and 80.2, respectively.

Protein processing:

Human NsG33 contains an N-terminal signal peptide sequence of 23 amino acids which is cleaved at the sequence motif ARA-GY. This signal peptide cleavage site is predicted by the SignalP method (Nielsen *et al.*,1997) and the output graph shown in FIG.1. A signal peptide cleavage site is found at a similar location in the mouse NsG33 (pos. 24) and rat NsG33 (pos. 16 or 21).

Proprotein processing:

General-type proprotein cleavage is predicted in human NsG33 (SEQ ID No 3) by the ProP method at pos. 127 with a score of 0.831, sequence motif 'WGPRERR-AL'. Similar, a cleavage site is predicted in mouse NsG33 (SEQ ID No 8) at pos. 128 with a score of 0.831, sequence motif 'WGPRERR-AL' and in rat NsG33 (SEQ ID No 13) at pos. 125 with a score of 0.831 and the sequence motif 'WGPRERR-AL'.

Protein function:

NsG33 belongs to the category of proteins acting as growth factors. This notion is supported by predictions by the ProtFun protein function prediction server (Jensen *et al.*, 2002 & 2003), which provides odds scores above 1 for exactly this type of category as shown in FIG. 2. The ProtFun method predicts protein function based on sequence-derived features as opposed to sequence similarity. Features which are important for discriminating between the 'growth factor' classes versus all other classes are: protein sorting potential, protein targeting potential, signal peptide potential, low complexity regions, secondary protein structure, number of negative residues and number of atoms (Jensen *et al.*, 2003).

The sequence identity calculations below have been made with the align0 program, using a BLOSUM50 matrix and gap penalties -12/-2.

Table 1 shows the % sequence identity between full length human NsG33 versus mouse and rat sequences.

Sequence	% id
human	-

mouse	80.3
rat	80.2

Table 2 shows the % sequence identity between human NsG33 versus mouse and rat sequences after removal of N-terminal signal peptide.

Sequence	% id
human	-
mouse	81.9
rat	79.6

References:

ProP: Prediction of proprotein convertase cleavage sites. *Peter Duckert, Søren Brunak and Nikolaj Blom.* Protein Engineering, Design and Selection: 17: 107-112, 2004

SignalP: Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Henrik Nielsen, Jacob Engelbrecht, Søren Brunak and Gunnar von Heijne, Protein Engineering 10, 1-6 (1997).

ProtFun: Ab initio prediction of human orphan protein function from post-translational modifications and localization features. L. Juhl Jensen, R. Gupta, N. Blom, D. Devos, J. Tamames, C. Kesmir, H. Nielsen, H. H. Stærfeldt, K. Rapacki, C. Workman, C. A. F. Andersen, S. Knudsen, A. Krogh, A. Valencia and S. Brunak. J. Mol. Biol., 319:1257-1265, 2002.

Prediction of human protein function according to Gene Ontology categories, L.J. Jensen, R. Gupta, H.H. Stærfeldt, S. Brunak, Bioinformatics, 19, 635-642 (2003).

align0 Optimal alignments in linear space. Myers, E.W. and Miller,W. Comput. Appl. Biosci., 4, 11-17 (1998).

Example 3, Gene-chip experiments

The human material comes from discarded tissue pieces obtained from electively terminated pregnancies using the regular vacuum aspiration technique. The collection of residual tissue for the study is approved by the Human Ethics Committee of the Huddinge University Hospital,

Karolinska Institute (Diary Nr. 259/00) and Lund University (970401), and is in accordance with the guidelines of the Swedish National Board of Health and Welfare (Socialstyrelsen), including an informed consent from the pregnant women seeking abortions. Recovered nervous tissue is micro-dissected within 2 hours of surgery and appropriate tissue fragments are further dissociated for cell isolation.

RNA isolation:

Human fetal tissue (8 weeks) was obtained in two rounds, both 8-weeks gestation age. Dissected VM and DM regions were used for total RNA isolation with good results and yields.

Total RNA was isolated with the Trizol extraction following the manufacturer's instruction (Invitrogen) from ventral and dorsal mesencephalic regions subdissected from human fetal tissue, 8 weeks gestational age. To concentrate RNA and to remove traces of chromosomal DNA, Rneasy columns combined with with the RNase-Free DNase Set are used following the manufacturer's instructions.

From 5 µg of total RNA, biotinylated cRNA is prepared and fragmented as described in Affymetrix protocols (GeneChip Expression Analysis, Technical Manual 2000) and hybridized (15 µg) to Affymetrix Human U133B GeneChips (containing approximately 22,000 genes) according to manufacturer's instructions. Scanned images are analyzed and converted to expression index values using the GenePublisher analysis software package (Knudsen S, Workman C, Sicheritz-Ponten T, Friis C. (2003) "GenePublisher: Automated analysis of DNA microarray data.", Nucleic Acids Res. 31(13):3471-6.).

Using Affymetrix U133 GeneChips the expression of human NsG33 was analysed (acc. 232269_x_at on U133 B and acc. 219051_x_at on U133 A GeneChip; Affymetrix, Inc., Santa Clara, Calif.). Expression of human NsG33 was observed in human 8-weeks fetal mesencephalon (midbrain) tissue samples indicating that human NsG33 may play a role in early fetal brain development. Expression of a growth factor in the human mesencephalon during embryo development is predictive of a possible therapeutic function in the treatment of Parkinson's Disease.

Example 4, obtaining a full length coding sequence

NsG33 was PCR amplified from an IMAGE clone (The I.M.A.G.E. Consortium: "An integrated molecular analysis of genomes and their expression", Lennon, Auffray, Polymeropoulos, and

Soares, [1996], Genomics 33: 151-152) obtained from RZPD, Berlin, Germany (RZPD clone ID: IRALp962D105Q2) using the following primers:

5' primer: 5'-GCGGATCCAGCGGTGGTGAGAGCCCCGAC-3'

3' primer: 5'-TATACTCGAGGCCCACCCTCCCTCCTACCAG-3'

Three identical PCR reactions were set up with 50 ng/µl of the RZPD clone as DNA template in a 50 µl reaction volume. A proofreading polymerase (pfu-turbo polymerase, Stratagene) was applied for the PCR amplification, with the following amplification profile: pre-denaturation step: 95°C, 1' followed by 35 3-step cycles: denaturation step: 95°C, 30"; annealing step: 57°C, 30"; elongation step: 72°C, 90". Then an elongation step: 72°C, 2' followed by cooling to 4°C.

PCR reactions wer epooled and the 988 bp NsG33 PCR fragment was agarose gel-purified and cut with BamHI and XhoI. The now 976 bp BamHI/XhoI-restricted NsG33 PCR fragment was gel-purified. Five µg of a lentiviral transfer vector, pHsCXW, (GenBank accession #: AY468486) was digested with BamHI and XhoI and the vector backbone was gel purified.

The BamHI/XhoI NsG33 PCR fragment was ligated into the BamHI and XhoI sites of the pHsCXW lentiviral transfer vector followed by transformation into XL1-B electrocompetent cells.

Example 5, Real Time PCR on NsG33

The tissues investigated for NsG33 expression were total RNA from Retina, Whole brain, Putamen, Substantia nigra, Ganglion, Fetal liver, Cerebellum, Whole brain, Fetal liver, Heart, Kidney, Lung, Placenta, Prostate, Salivary gland, Skeletal muscle, Spleen, Testis, Thymus, Trachea, Uterus, Colon, Small intestine, Spinal cord, Stomach, Pancreas, Fetal brain.

First strand cDNA was prepared from total RNA using Superscript II Reverse Transcriptase (Life Technologies) and a HT11V primer using standard procedures. For real-time PCR expression analysis product from the Reverse Transcription equivalent to 20 ng of each RNA, was used as template in real-time PCR reactions.

Real-time PCR was performed in an Opticon-2 thermocycler (MJ Research), using LightCycler-FastStart DNA Master SYBR Green I kit (Roche). Studies were carried out in duplicates using primers 5'-CCAGCGACTTCGTAATTCAC-3' (5' primer) and 5'-AGCCCATGAAGAGGAAGG-3' (3' primer). For Real-Time PCR, a standard curve was prepared by serial dilution of a gel-purified PCR product, prepared using the above primers. The standard curve was used to verify

that crossing-point values (CT) of all samples were within the exponential range of the PCR reaction and to calculate final expression levels. All RT-PCR amplifications were performed in a total volume of 10 µl containing 3 mM $MgCl_2$, 12 % sucrose and 1x reaction buffer included in the LightCycler kit. PCR cycling profile consisted of a 10 minutes pre-denaturation step at 98 °C and 35 three-step cycles at 98 °C for 10 seconds, at 62 °C for 20 seconds and at 72 °C for 20 seconds. Following the extension step of each cycle, a plate reading step was added (80 °C, 2 seconds) to quantify the newly formed PCR products. The specificity of the amplification reaction was determined by performing a melting curve analysis of the PCR fragments by slowly raising the temperature from 52 °C to 95 °C with continuous data acquisition.

For normalization purposes, all cDNAs were subjected to real-time PCR using primers for β_2-microglobulin (B2M, 5'-TGTGCTCGCGCTACTCTCTC-3' and 5'-CTGAATGCTCCACTTTTTCAATTCT-3'). Standard curves for β_2-microglobulin were prepared similar to NsG33. Housekeeping gene real-time PCR was done using the same kit as for the target gene, except that optimal annealing temperatures were used for the housekeeping gene.

Housekeeping expression pattern was determined from the respective standard curves and the relative expression levels were used to normalize expression levels of the target genes in the tissues that were analyzed. Following normalization with the β_2-microglobulin, relative expression levels of the target gene were calculated using the tissue with the lowest expression as a reference. Results normalised with respect to β_2-microglobulin should be interpreted with caution, since β_2-microglobulin may not be expressed at the same leve in all tested tissues.

Analysis of total RNA samples (shown in Figures 4 A and B)

<u>High expression (C(T) values< 22)</u>

Putamen, Substantia Nigra, Spinal Cord

<u>Intermediate expression (22< C(T) values< 24)</u>

Whole brain, Cerebellum, retina, DRG

<u>Low expression (24< C(T) values< 26)</u>

Heart, Kidney, lung, prostate, salivary gland, skeletal muscle, testis, stomach, pancreas, fetal brain

<u>Very low or no expression (C(T) values >26)</u>

Fetal Liver, Placenta, thymus, trachea, spleen, uterus, colon, small intestine

Based on the tissue specific expression, and the fact that NsG33 is predicted to be a secreted growth factor (see example 2), NsG33 is contemplated for use in treating disorders of the

nervous system in general (based on the nervous-system specific expression), in particular Parkinson's disease (based on the expression in substantia nigra), Huntingtons disease (based on expression in Putamen), cerebellar disorders (based on expression in cerebellum), Spinal Cord injury and ALS (based on expression in the spinal cord), peripheral neuropathies (based on expression in dorsal root ganglion), retinopathies (based on expression in retina). The function for the various indications can be verified in in vitro and in vivo assays as described below.

Example 6: Testing for general neuroprotective effect (PC-12 assay)

Generation of virus stock:

NsG33 coding sequence is subcloned into pHsCXW using appropriate restriction sites as described in Example 4. To generate virus stocks, the resulting lentiviral transfer vector is cotransfected into 293T cells with two helper plasmids (pMD.G and pBR8.91) providing the necessary viral genes, gag-pol and env, respectively, in trans. Briefly, $2x10^6$ 293T cells are seeded in each of 20 T75 culture flasks. The next day, each T75 flask is transfected with 15 µg ppBR8.91, 5 µg pMD.G and 20 µg of transfer vector using Lipofectamine+ following the manufacturer's instructions (Invitrogen). Virus-containing medium is harvested 2-3 days after the transfection and filter-sterilized through a 0.45 µm cellulose acetate or polysulphonic filter. The virus is pelleted by double ultracentrifugation at 50,000xg for 90 minutes at 4°C and then resuspended in DMEM medium. Virus is titrated using a reverse transcription (RT) assay (Current Protocols in Molecular Biology, Editors: Ausubel et al., Willey). The number of transducing units (TU)/ml is calculated from the resulting RT activity and frequency of fluorescent cells obtained by transduction of 293T cells with an equivalent GFP lentivirus. The virus stock is stored in aliquots at –80°C until use.

Transduction of PC12 cells:

PC12 cells are cultured in Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/l glucose and glutamax (Life Technologies #32430-027) with 7.5% donor horse serum (Life Technologies #16050-098) and 7.5% FBS (Life Technologies # 10099-141) in the presence of 5% CO_2 at 37°C. Medium is changed every 2-3 days and cells are subcultured 1:3 - 1:6 twice a week by tapping the flask and dispensing into new flasks. The day before transduction, cells are seeded in 6-well plates coated with collagen. Virus is added from the stock solution to 1ml cell culture medium together with or without 5 µg/ml (final conc.) polybrene. The virus is incubated with the cells for at least 3 hours in a CO_2 incubator. GFP retrovirus is added to a parallel culture to estimate transduction efficiency and to serve as control.

Effect on PC12 differentiation:
Cultures in 6-well plates are followed and scored for the number of neurite bearing cells after 2-5 days.

Effect on PC12 survival:
Transduced cells from 6-well plates are reseeded in 96-well plates coated with collagen in culture medium. The following day, medium is changed to serum-free DMEM and cell viability is measured after 24-72 hr using the MTS assay following the manufacturer's instructions (Promega).

A positive effect in either the neurite outgrowth and/or the survival assay is indicative of a potential therapeutic effect of the encoded secreted protein in treating neurodegenerative disorders.

Example 7: Protection of cerebellar granule cells from glutamate toxicity

Testing for survival effects is carried out by transducing cultures of cerebellar granule cells that subsequently is exposed to toxic concentrations of glutamate essentially as described (Daniels and Brown, 2001; J. Biol. Chem. 276: 22446-22452).

Cerebellar granule neurons (CGN) are dissected from 7-8 days old mouse pups. Cells are dissociated from freshly dissected cerebella by enzymatic disruption in the presence of trypsin and DNase and then plated in poly-D-lysine-precoated 24-well plates (Nunc) at a density of 1-2 × 10^6 cells/cm^2 in DMEM medium supplemented with 10% heat-inactivated fetal calf serum. Cells are cultured at 37°C in a humidified atmosphere and Cytosine arabinoside (10 µM) is added to the culture medium after 24 hr to arrest the growth of non-neuronal cells.

Cultures are transduced with an NsG33 containing lenti-virus prepared as described in Example 7 on DIV1 by the addition of virus stock solution to DMEM medium containing 10 % Fetal bovine serum and 4 µg/ml Polybrene. Parallel control cultures are transduced with a Green Fluorescent Protein (GFP) lentivirus. Five hours after the transduction, medium is replaced with medium preconditioned on CGNs.

At DIV5, glutamate (0.1-1 mM) is added the the culture and after two additional days cell survival is assayed using the MTT assay. The extent of MTT reduction to formazane is

measured spectrophotometrically at 570 nm, in a lambda-2 spectrophotometer. Briefly, culture medium is removed, and cells are washed in sodium saline solution (140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2 . 6H_2O$, 1 mM NaH_2PO_4, 1.5 mM $CaCl_2$, 5.6 mM glucose, 20 mM HEPES, pH 7.4). MTT (final concentration 0.5 mg/ml), preparted just before using and maintained in the dark in sodium saline solution, is then added to the cells. After a 3 h incubation at 37°C, an euqal volume of acid-isopropanol (0.04 M HCl in isopropanol) is added and mixed thoroughly at room temperature until all formazan crystals were dissolved. Cell viability is expressed as a percentage of the optical densitiy of control cells. Parallel cultures are left untreated.

This assay can be considered as a general assay for testing of protection against excitotoxic damage as well as an assay predictive for factors with therapeutic potential in the treatment of cerebellar disorders.

Example 8, Protection of cerebellar granule cells from apoptosis induced by potassium deprivation

Testing for survival effects is carried out by transducing cerebellar granule cells deprived of potassium essentially as described (Nomura et al., 2001; Dev. Neurosci. 23: 145-152).

Cerebellar granule neurons (CGN) are dissected from 8-d-old Sprague-Dawley rat pups. Cells are dissociated from freshly dissected cerebella by enzymatic disruption in the presence of trypsin and DNase and then plated in poly-L-lysine-precoated 96-well plates (Nunc) at a density of 3.5×10^5 cells/cm^2 in Eagle's basal medium containing 25 mM KCl and supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine. Cells are cultured at 37°C in a humidified atmosphere and Cytosine arabinoside (10 µM) is added to the culture medium after 24 hr to arrest the growth of non-neuronal cells.

Cultures are transduced with an NsG33 containing lenti-virus prepared as described in Example 6 ["Testing in PC12 cells"] on DIV1 by the addition of virus stock solution to DMEM medium containing 10 % Fetal bovine serum and 4 µg/ml Polybrene. Parallel control cultures are transduced with a GFP lentivirus. Five hours after the transduction, medium is replaced with medium preconditioned on CGNs.

At DIV2, apoptosis is induced in *immature* cultures by switching the cells to serum-free medium containing 5 mM KCl, while the untreated cells received conditioned medium containing 25 mM KCl. Survival is measured on DIV3, using the MTS assay.

At DIV8, apoptosis is induced in *differentiated (neuronal)* cultures by switching the cells to serum-free medium containing 5 mM KCl, while the untreated cells received conditioned medium containing 25 mM KCl. Survival is measured after 24-72 hr, using the MTS assay.

The MTS assay is carried out using the The CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay (Promega) following the manufacturer's instructions.

This assay can be considered as a general assay for neuroprotective effects as well as an assay predictive for factors with therapeutic potential in the treatment of cerebellar disorders.

Example 9, Effect on DRG cultures

Preparation of conditioned media from transduced ARPE-19 cells. To transduce ARPE-19 cells with a lentivirus containing cDNA encoding the NsG33 gene, cells are plated at a density of 1 $x10^5$ cells/well in a 6-well plate in DMEM/F12 medium supplemented with 10 % Fetal Bovine Serum. Next day virus is added from the stock solution to the cell culture medium together with 5 µg/ml (final conc.) polybrene. The virus is incubated with the cells overnight in a CO_2 incubator. GFP lentivirus is added to a parallel culture. The next day, cultures are changed to serum-free UltraCULTURE medium (1 ml/well) and conditioned media are harvested after two additional days of incubation.

Isolation and culture of P1 DRG cells. DRGs from all spinal levels are removed from P1 (post-natal day 1) Sprague-Dawley. Tissues are enzymatically dissociated in 125-250 U/ml type 1 collagenase (Worthington, Freehold, N.J.) at 37°C for 30 minutes. Samples are triturated with fire-polished Pasteur pipettes and filtered though 70 µm sterile mesh to produce single cell suspensions. Cells are pre-plated on non-coated tissue-culture-ware dishes for 2 hours to remove non-neuronal cells. Non adherent cells are plated at 15,000 cells/well in 24-well tissue culture dishes that had been coated with poly-d-ornithine (Life Technologies) and laminin (Collaborative Biomedical). Negative controls are cultured in UltraCULTURE serum-free media, (BioWhittaker, Walkersville, MD) containing 2.5µg/ml sheep-neutralizing anti-NGF pAb (Chemicon, Temecula, CA). NGF-treated positive controls lacked the neutralizing anti-NGF pAb. Different dilutions of conditioned medium collected from NsG33-transduced or GFP-transduced ARPE-19 cells are added to the cultures after centrifugation and filtering through a 0.4 µm sterilfilter. Cultures are fed every second day by replacing the media.

Immunocytochemistry. After seven days in culture, cells are fixed in 4% formaldehyde in PBS for 10 minutes at room temperature. Cells are pre-blocked in 4% goat serum, 0.1% NP40 for 30 minutes at room temperature and then incubated with mouse anti-ßIII tubulin (1:100) overnight at 4°C. After rinsing in pre-block solution, the cultures are incubated with a secondary Cy-3 coupled anti-murine antibody for 1 hour at room temperature. Following a final rinse in pre-block solution, cells from a strip through the middle of each well are counted using fluorescence optics. All ßIII-tubulin positive cells are scored as neurons and survival is determined by the number of neurons counted per well. All antibodies are diluted in pre-block solution.

Interpretation of results

Protective effects in this assay indicates therapeutic potential in peripheral neuropathies and neuropathic pain

Example 10, Effect on Motoneuron cultures

Testing for survival effects on motoneuron cultures is carried out using NsG33 containing lentivirus essentially as described in Cisterni et al. 200 (J. Neurochem. 74, 1820-1828). Briefly, ventral spinal cords of embryonic day 14.5 (E14.5) Sprague Dawley rat embryos are dissected and dissociated. Motoneurons are purified using a protocol based on the immunoaffinity purification of motoneurons with antibodies against the extracellular domain of the neurotrophin receptor, p75, followed by cell sorting using magnetic microbeads (Arce et al. 1999). Purified motoneurons are seeded on 4-well tissue culture dishes precoated with polyornithine/laminin at density of 500 cells per well. Culture medium is Neurobasal culture medium (Life Technologies) supplemented with the B27 supplement (Life Technologies), horse serum (2% v/v), L-glutamine (0.5 mM), and 2-mercaptoethanol (25 µM). L-Glutamate (25 µM) is added to the medium during the first 4 d of culture and subsequently omitted.

Motoneurons cultured for 16 h are transduced with an NsG33 containing lenti-virus prepared as described above by the addition of virus stock solution to the culture medium (corresponding to MOI=4). Parallel control cultures are transduced with a GFP lentivirus. Eight hours after the transduction, medium is replaced with fresh medium (DIV1).

Motoneuron survival is quantified at DIV3 by counting the number of large phase-bright neurons with long axonal processes in a predetermined area of 1.5 cm^2 in the center of duplicate dishes.

Interpretation of results

Protective effects in this assay indicates therapeutic potential in motoneuron diseases including ALS, Spinal Cord injury, SMA (spinal muscular atrophy), DMD (Duchenne muscular dystrophy).

Example 11: Bioassay for dopaminergic neurotrophic activities.

Culture conditions:

Dissociated mesencephalic cell cultures are prepared as previously described (Friedman and Mytilineou 1987 Neurosci. Lett. 79:65-72), with minor modifications. Briefly, rostral mesencephalic tegmentum from brains of Sprague-Dawley rat embryos, at the 13th-16th day of gestation, are dissected under the microscope in sterile conditions, collected in Ca^{2+} - and Mg^{2+} -free Dulbecco's phosphate buffered saline (Gibco, Gaithersburg, Md.) and dissociated mechanically by mild trituration. The cells are plated in 100 µl per well onto 16-mm diameter tissue culture wells (Falcon, Lincoln Park, N.J., 24-well plate) containing 400 µl medium to give a density of 2.5-3.5x10^5 cells per well. The culture wells have been previously exposed to 0.1 mg/ml solution of poly L-ornithine in 10 mM sodium borate, pH 8.4, for 3 hours at 37°C., washed 3 times in milli-Q H_2O and once in Earle's balanced salt solution (Gibco). The feeding medium (10/10) consists of minimal essential medium (MEM, Gibco) supplemented with glucose (33 mM), sodium bicarbonate (24.5 mM), glutamine (2 mM), HEPES (15 mM), penicillin G (5 U/ml), streptomycin (5 µg/ml), 10% heat-inactivated fetal calf serum (Gibco) and 10% heat inactivated horse serum (Gibco). The cultures are kept at 37°C in a water-saturated atmosphere containing 6.5% CO_2. After 3 hours, when most of the cells have adhered to the bottom of the well, the medium is replaced with 500 µl of fresh medium. At this time, a serial dilution of the sample to be assayed for dopaminergic neurotrophic activity (conditioned medium) is added to each well in duplicate and the plates are incubated in the 37°C incubator. After a week, the cultures are treated for 24 hours with fluorodeoxyuridine (13 µg/ml) and uridine (33 µg/ml) to prevent excessive glial proliferation and subsequently fed with the above medium without fetal calf serum. The feeding medium is changed weekly.

Alternatively, chemically defined serum-free medium is used in which serum is replaced by a mixture of proteins, hormones and salts. The defined medium (DM) consists of a mixture of MEM and F12 nutrient mixture (both Gibco, 1:1; vol/vol) with glucose (33 mM), glutamine (2 mM) $NaHCO_3$ (24.5 mM), HEPES (15 mM), supplemented with transferrin (100 µg/ml), insulin (25 µg/ml), putrescine (60 µM), progesterone (20 nM), sodium selenite (30 nM), penicillin G (5 U/ml) and streptomycin (5 µg/ml). The osmolarity of the DM is adjusted to 325 by the addition of milli-Q H_2O. (110-125 ml H_2O/l).

The functional status of the dopaminergic neurons may be assayed in these cultures by measuring dopamine uptake through specific "scavenger" transporters in the dopaminergic neurons and by counting the number of neurons positive for the dopamine synthetic enzyme tyrosine hydroxylase using immunohistochemistry as described in Karlsson et al, 2002, Brain Res. 2002 Nov 15;955(1-2):268-80.

Sample preparation:

Prior to being assayed for dopaminergic neurotrophic activity in the mesencephalic cell cultures, all the samples of conditioned medium are desalted as follows. One hundred µl of the medium 10/10 (as a carrier) is added to a Centricon-10 (Amicon) and allowed to sit for 10 minutes. Aliquots of the sample to be assayed are added to the Centricon, followed by 1 ml of Dulbecco's high glucose Modified Eagle medium, without bicarbonate, but containing 10 mM HEPES, pH 7.2 (solution A), and centrifuged at 5,000Xg for 70 minutes. The retentate (about 0.1 ml) is brought back to 1.1 ml with fresh solution A and reconcentrated twice. The sample is filtered through a 0.11 µm Ultrafree-MC sterile Durapore unit (Millipore, Bedford Mass.) prior to being added to the culture well.

^{3}H-dopamine uptake:

Uptake of tritiated dopamine (^{3}H-DA) is performed in cultures at day 6 or day 7 as described previously (Friedman and Mytilineou (1987) Neurosci. Lett. 79:65-72) with minor modifications, and all the solutions are maintained at 37°C. Briefly, the culture medium is removed, rinsed twice with 0.25 ml of the uptake buffer which consists of Krebs-Ringer's phosphate buffer, pH 7.4, containing 5.6 mM glucose, 1.3 mM EDTA, 0.1 mM ascorbic acid and 0.5 mM pargyline, an inhibitor of monoamine oxidase. The cultures are incubated with 0.25 ml of 50 nM ^{3}H-DA (New England Nuclear, Boston, Mass. sp. act 36-37 Ci/mmol) for 15 minutes at 37°C. ^{3}H-DA uptake is stopped by removing the incubation mixture and cells are then washed twice with 0.5 ml of the uptake buffer. In order to release ^{3}H-DA from the cells, the cultures are incubated with 0.5 ml of 95% ethanol for 30 min at 37°C., and then added to 10 ml of EcoLite (ICN, Irvine, Calif.) and counted on a scintillation counter. Blank values are obtained by adding to the uptake buffer 0.5 mM GBR-12909 (RBI), a specific inhibitor of the high-affinity uptake pump of the dopamine neurons (Heikkila et al. 1984 Euro J. Pharmacol. 103:241-48).

An increase in the number of TH positive neurons and/or an increase in 3H-dopamine uptake compared to a control treatment is an indication of a possible function of NsG33 in the treatment of Parkinson's disease.

Example 12: Assessment of neuroprotection of nigral dopamine neurons *in vivo* in the instrastriatal 6-OHDA lesion model.

VSV-G pseudotyped (rLV) vectors are produced as described previously (Zufferey et al., 1997; Rosenblad et al., 2000a). Briefly, the transfer plasmids pHR'CMV-W carrying the cDNA for green fluorescent protein (GFP) or NsG33 is co-transfected with the helper plasmids pMD.G and pCMVDR8.91 into 293T cells. Virion containing supernatants are collected on days 2 and 3 after transfection and concentrated at 116 000 g by ultracentrifugation. The titer of rLV-GFP vector stock is 1.1 x 10^8 TU/ml as determined by serial dilution of the concentrated supernatant on 293T cells. The viral particle titre is determined for rLV-NsG33 and rLV-GFP virus stocks using an RNA slot blot technique as described previously (von Schwedler et al., 1993) and from the ratio between TU and viral particle titre obtained for rLV-GFP, the titre of the rLV-NsG33 vector is estimated to be 1.2 x 10^8 TU/ml

All work involving experimental animals are conducted according to the guidelines set by the Ethical Committee for Use of Laboratory Animals at Lund University. Animals are housed in 12:12 hour light/dark cycle with access to rat chow and water. Female Sprague Dawley rats (~220g by the time of surgery) are used. For stereotaxic surgery animals are anesthetized using halothane and a total of two microliters rLV-GFP (n=8) or rLV-NsG33 of a 1:2 viral stock (1.0-1.2 x 10^5 TU) are injected into two tracts in the right striatum at the following coordinates: (1) AP = +1.0 mm, ML = -2.6 mm, DV = -5.0 and –4.5 mm, Tb = 0.0 and (2) AP = 0.0 mm, ML = -3.7 mm, DV = -5.0 and –4.5 mm, Tb = 0.0. After two weeks the animals are again anesthetized and placed in the stereotaxic frame. An injection of 6-hydroxydopamine (20 µg [calculated as free base] per 3 µl vehicle [saline with 0.2% ascorbic acid]) is made into the right striatum at the following coordinates: AP = +0.5 mm, ML = -3.4 mm, DV = -5.0 and –4.5 mm, Tb = 0.0.

At four weeks post-lesion the animals are deeply anesthetized with pentobarbital (70 mg/kg, Apoteksbolaget, Sweden), and transcardially perfused with 50ml saline at room temperature, followed by 200 ml ice-cold phosphate-buffered 4% paraformaldehyde (pH 7.2-7.4). The brains are postfixed for 3-6 hours in the same fixative, transferred to 30% sucrose for 24 hours and cut into 6 series of 40 µm thick sections on a freezing microtome.

Immunohistochemistry for detection of tyrosine hydroxylase-immunoreactive, in the substanita nigra is performed as described previously (Rosenblad et al., 2000a). The number of TH-IR and VMAT-IR nigral neurons is assessed by counting under microscope all immunoreactive neurons lateral to the medial terminal nucleus of the accessory optic tract in three consecutive sections through the SN, as described previously (Sauer & Oertel, 1994).

An increase in the number of TH-IR compared to the GFP control is a strong indication of a function in the treatment of Parkinson's disease. An increase in the number of VMAT-IR further strengthens the conclusion.

CLAIMS

1. An isolated polypeptide for medical use, said polypeptide comprising an amino acid sequence selected from the group consisting of:
   a) the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 8, 9, 10, 13, 14, 15, 19, 20, 21, 22, 23, and 24;
   b) a sequence variant of the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 8, 9, 10, 13, 14, 15, 19, 20, 21, 22, 23, and 24, wherein the variant has at least 70% sequence identity to said SEQ ID No.; and
   c) a biologically active fragment of at least 50 contiguous amino acids of any of a) through b).

2. The polypeptide of claim 1 that is a naturally occurring allelic variant of the sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 8, 9, 10, 13, 14, 15, 19, 20, 21, 22, 23, and 24.

3. The polypeptide of claim 2, wherein the allelic variant comprises an amino acid sequence that is the translation of a nucleic acid sequence differing by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID No. 1, 2, 6, 7, 11, 12, 16, 17, and 18.

4. The polypeptide of claim 1 that is a variant polypeptide described therein, wherein any amino acid specified in the chosen sequence is changed to provide a conservative substitution.

5. The polypeptide of claim 1, wherein the signal peptide has been replaced by a heterologous signal peptide.

6. The polypeptide of claim 1, having at least 70% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID No. 5, 10, and 15 more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence selected from the group consisting of SEQ ID No. 5, 10, and 15.

7. The polypeptide of claim 1, having at least 70% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID No. 4, 9, and 14, more

preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence selected from the group consisting of SEQ ID No. 4, 9, and 14.

8. The polypeptide of claim 1, having at least 70% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID No. 3, 8, and 13, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence selected from the group consisting of SEQ ID No. 3, 8, and 13.

9. The polypeptide of claim 1, having at least 70% sequence identity to a protein having a sequence selected from the group consisting of SEQ ID No. 19, 20, 21, 22, 23, and 24, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence selected from the group consisting of SEQ ID No. 19, 20, 21, 22, 23, and 24.

10. The polypeptide of claim 1, having at least 70% sequence identity to the protein having the sequence of SEQ ID No. 3, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence of SEQ ID No. 3.

11. The polypeptide of claim 1, having at least 70% sequence identity to the protein having the sequence of SEQ ID No. 4, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence of SEQ ID No. 4.

12. The polypeptide of claim 1, having at least 70% sequence identity to the protein having the sequence of SEQ ID No. 5, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence of SEQ ID No. 5.

13. The polypeptide of claim 1, having at least 70% sequence identity to the protein having the sequence of SEQ ID No. 19, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence of SEQ ID No. 19.

14. The polypeptide of claim 1, having at least 70% sequence identity to the protein having the sequence of SEQ ID No. 22, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence of SEQ ID No. 22.

15. The polypeptide of claim 1, wherein the fragment is selected from the group consisting of:

i) AA_{30}-AA_{288} of SEQ ID No 3, and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{25}-AA_{293} of SEQ ID No 3;

ii) AA_{28}-AA_{286} of SEQ ID No 13 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{23}-AA_{291} of SEQ ID No 13;

iii) AA_{31}-AA_{289} of SEQ ID No 8 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{26}-AA_{294} of SEQ ID No 8; and

iv) variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 20 of the amino acid residues in the sequence are so changed.

16. The polypeptide of claim 1, selected from the group consisting of:

i) AA_{171}-AA_{288} of SEQ ID No 3, and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{165}-AA_{288} of SEQ ID No 3;

ii) AA_{169}-AA_{286} of SEQ ID No 13 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{164}-AA_{291} of SEQ ID No 13;

iii) AA_{172}-AA_{289} of SEQ ID No 8 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, i.e. up to AA_{167}-AA_{294} of SEQ ID No 8;

iv) variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed.

17. The polypeptide of claim 1, wherein the fragment is selected from the group consisting of

i) AA_{30}-AA_{118} of SEQ ID No 3, and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{25}-AA_{123} of SEQ ID No 3;

ii) AA_{28}-AA_{116} of SEQ ID No 13 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{23}-AA_{121} of SEQ ID No 13;

iii) AA_{31}-AA_{119} of SEQ ID No 8 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{26}-AA_{124} of SEQ ID No 8; and

iv) variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed.

18. The polypeptide of any of the preceding claims 15 to 17, wherein the changed amino acids are selected from those designated as unconserved, weakly conserved or strongly conserved in Figure 3.

19. The polypeptide of any of the preceding claims, being capable of forming at least one intramolecular cystin bridge.

20. The polypeptide of any of the preceding claims, comprising a dimer of NsG33 linked through an intermolecular cystin bridge.

21. The polypeptide according to any of the preceding claims, further comprising an affinity tag, such as a polyhis tag, a GST tag, a HA tag, a Flag tag, a C-myc tag, a HSV tag, a V5 tag, a maltose binding protein tag, a cellulose binding domain tag.

22. An isolated nucleic acid molecule for medical use comprising a nucleic acid sequence encoding a polypeptide or its complementary sequence, said polypeptide comprising an amino acid sequence selected from the group consisting of:

a) the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 8, 9, 10, 13, 14, 15, 19, 20, 21, 22, 23, and 24;

b) a sequence variant of the amino acid sequence selected from the group consisting of SEQ ID No. 3, 4, 5, 8, 9, 10, 13, 14, 15, 19, 20, 21, 22, 23, and 24, wherein the variant has at least 70% sequence identity to said SEQ ID No.; and

c) a biologically active fragment of at least 50 contiguous amino acids of any of a) through b).

23. The nucleic acid molecule of claim 22, wherein the nucleic acid molecule comprises the nucleotide sequence of a naturally occurring allelic nucleic acid variant.

24. The nucleic acid molecule of claim 22 that encodes a variant polypeptide, wherein the variant polypeptide has the polypeptide sequence of a naturally occurring polypeptide variant.

25. The nucleic acid molecule of claim 22, wherein the nucleic acid molecule differs by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID No. 1, 2, 6, 7, 11, 12, 16, 17, and 18.

26. The nucleic acid molecule of claim 22, wherein the encoded polypeptide has at least 70% sequence identity to a sequence selected from the group consisting of SEQ ID No. 5, 10, and 15 more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence selected from the group consisting of SEQ ID No. 5, 10, and 15.

27. The nucleic acid molecule of claim 22, wherein the encoded polypeptide has at least 70% sequence identity to a sequence selected from the group consisting of SEQ ID No. 4, 9, and 14, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence selected from the group consisting of SEQ ID No. 4, 9, and 14.

28. The nucleic acid molecule of claim 22, wherein the encoded polypeptide has at least 70% sequence identity to a sequence selected from the group consisting of SEQ ID No. 3, 8, and 13, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence selected from the group consisting of SEQ ID No. 3, 8, and 13.

29. The nucleic acid molecule of claim 22, wherein the encoded polypeptide has at least 70% sequence identity to a sequence selected from the group consisting of SEQ ID No. 19, 20, 21, 22, 23, and 24, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein

having the sequence selected from the group consisting of SEQ ID No. 19, 20, 21, 22, 23, and 24.

30. The nucleic acid molecule of claim 22, wherein the encoded polypeptide has at least 70% sequence identity to SEQ ID No. 3, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence of SEQ ID No. 3.

31. The nucleic acid molecule of claim 22, wherein the encoded polypeptide has at least 70% sequence identity to SEQ ID No. 4, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence of SEQ ID No. 4.

32. The nucleic acid molecule of claim 22, wherein the encoded polypeptide has at least 70% sequence identity to SEQ ID No. 5, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence of SEQ ID No. 5.

33. The nucleic acid molecule of claim 22, wherein the encoded polypeptide has at least 70% sequence identity to SEQ ID No. 19, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence of SEQ ID No. 19.

34. The nucleic acid molecule of claim 22, wherein the encoded polypeptide has at least 70% sequence identity to SEQ ID No. 22, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a protein having the sequence of SEQ ID No. 22.

35. The nucleic acid molecule of claim 22, wherein the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of

a) the nucleotide sequence selected from the group consisting of SEQ ID No. 1, 2, 6, 7, 11, 12, 16, 17, and 18;

b) a nucleotide sequence having at least 70% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID No. 1, 2, 6, 7, 11, 12, 16, 17, and 18;

c) a nucleic acid sequence of at least 150 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID No. 1, 2, 6, 7, 11, 12, 16, 17, and 18;

c) the complement of a nucleic acid capable of hybridising with nucleic acid having the sequence selected from the group consisting of SEQ ID No.: 1, 2, 6, 7, 11, 12, 16, 17, and 18 under conditions of high stringency; and

d) the nucleic acid sequence of the complement of any of the above.

36. The nucleic acid molecule of claim 22, comprising a nucleotide sequence having at least 70% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID No. 2, 7, 12, 16, 17, and 18, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a nucleic acid having the sequence of SEQ ID No. 2, 7, 12, 16, 17, or 18.

37. The nucleic acid molecule of claim 22, comprising a nucleotide sequence having at least 70% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID No. 16, 17, and 18, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a nucleic acid having the sequence of SEQ ID No. 16, 17, or 18.

38. The nucleic acid molecule of claim 22, comprising a nucleotide sequence having at least 70% sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID No. 2 and 16, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a nucleic acid having the sequence of SEQ ID No. 2 and 16.

39. The nucleic acid molecule of claim 22, having at least 70% sequence identity to the nucleic acid molecule having the sequence of SEQ ID No. 1, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a nucleic acid having the sequence of SEQ ID No. 1.

40. The nucleic acid molecule of claim 22, having at least 70% sequence identity to the nucleic acid molecule having the sequence of SEQ ID No. 2, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a nucleic acid having the sequence of SEQ ID No. 2.

41. The nucleic acid molecule of claim 22, having at least 70% sequence identity to the nucleic acid molecule having the sequence of SEQ ID No. 6, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a nucleic acid having the sequence of SEQ ID No. 6.

42. The nucleic acid molecule of claim 22, having at least 70% sequence identity to the nucleic acid molecule having the sequence of SEQ ID No. 7, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a nucleic acid having the sequence of SEQ ID No. 7.

43. The nucleic acid molecule of claim 22, having at least 70% sequence identity to the nucleic acid molecule having the sequence of SEQ ID No. 11, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a nucleic acid having the sequence of SEQ ID No. 11.

44. The nucleic acid molecule of claim 22, having at least 70% sequence identity to the nucleic acid molecule having the sequence of SEQ ID No. 12, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a nucleic acid having the sequence of SEQ ID No. 12.

45. The nucleic acid molecule of claim 22, having at least 70% sequence identity to the nucleic acid molecule having the sequence of SEQ ID No. 16, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a nucleic acid having the sequence of SEQ ID No. 16.

46. The nucleic acid molecule of claim 22, having at least 70% sequence identity to the nucleic acid molecule having the sequence of SEQ ID No. 17, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a nucleic acid having the sequence of SEQ ID No. 17.

47. The nucleic acid molecule of claim 22, having at least 70% sequence identity to the nucleic acid molecule having the sequence of SEQ ID No. 18, more preferably at least 75%, more preferably at least 80%, more preferably at least 95%, more preferably at least 98%, more preferably a nucleic acid having the sequence of SEQ ID No. 18.

48. The nucleic acid molecule of claim 22, being codon optimised for expression in E. coli, Chinese Hamster, Baby Hamster, Yeast, insect and/or fungus.

49. The nucleic acid molecule of claim 22, wherein the nucleic acid molecule is a shuffled variant between SEQ ID No 2, and 7 and/or 12.

50. A vector comprising the nucleic acid molecule of any of the preceding claims 22 to 49.

51. The vector of claim 50, further comprising a promoter operably linked to the nucleic acid molecule.

52. The vector of claim 51, wherein the promoter is selected from the group consisting of: CMV, human UbiC, JeT, RSV, Tet-regulatable promoter, Mo-MLV-LTR, Mx1, EF-1alpha.

53. The vector of claim 50 or 51, being selected from the group consisting of vectors derived from the Retroviridae family including lentivirus, HIV, SIV, FIV, EAIV, CIV.

54. The vector of claim 50 or 51, being selected from the group consisting of alphavirus, adenovirus, adeno associated virus, baculovirus, HSV, coronavirus, Bovine papilloma virus, Mo-MLV, preferably adeno associated virus.

55. An isolated host cell treansformed or transduced with the vector of any of the claims 50 to 54.

56. The host cell of claim 55, being selected from the group consisting of E. coli, Yeast, Saccharomyces cerevisiae, Aspergillus, Sf9 insect cells.

57. The host cell of claim 55, being selected from the group consisting of mammalian cells, such as human, feline, porcine, simian, canine, murine, rat, mouse and rabbit.

58. The host cell of claim 57, being selected from the group consisting of immortalised retinal pigmented epithelial cells, such as ARPE-19 cells, immortalised human fibroblasts, and immortalised human astrocytes.

59. The host cell of claim 58, being attached to a matrix.

60. The host cell of claim 57, being selected from the group consisting of stem cells, including human neural stem or precursor cells, human glial stem or precursor cells, and foetal stem cells.

61. The host cell of claim 57, being selected from the group consisting of CHO, CHO-K1, HEI193T, HEK293, COS, PC12, HiB5, RN33b, BHK cells.

62. A packaging cell line capable of producing an infective virus particle, said virus particle comprising a Retroviridae derived genome comprising a 5' retroviral LTR, a tRNA binding site, a packaging signal, a promoter operably linked to a polynucleotide sequence encoding the polypeptide of any of the claims 1 to 21, an origin of second strand DNA synthesis, and a 3' retroviral LTR.

63. The packaging cell line of claim 61, wherein the genome is lentivirally derived and the LTRs are lentiviral.

64. An implantable biocompatible cell device, the device comprising:

i) a semipermeable membrane permitting the diffusion of a protein as defined by any of the preceding claims 1 to 21 and/or a virus vector; and

ii) a composition of cells according to any of the claims 55 to 61 or a packaging cell line according to any of the claims 62 to 63.

65. The device of claim 64, wherein the semipermeable membrane is immunoisolatory.

66. The device of claim 64, wherein the semipermeable membrane is microporous.

67. The device of claim 64, wherein the device further comprises a matrix disposed within the semipermeable membrane.

68. The device of claim 64, wherein the device further comprises a tether anchor.

69. The device of claim 64, wherein said device comprises a core comprising living packaging cells that secrete a viral vector for infection of a target cell, wherein the viral vector is a retrovirus, the vector comprising a heterologous gene encoding a polypeptide according to any of claims 1 to 21, operably linked to a promoter that regulates the expression of said polypeptide in the target cell; and an external jacket surrounding said

core, said jacket comprising a permeable biocompatible material, said material having a porosity selected to permit passage of retroviral vectors of approximately 100 nm diameter thereacross, permitting release of said viral vector from said capsule.

70. The device of claim 69, wherein the core additionally comprises a matrix, the packaging cells being immobilized by the matrix.

71. The device of claim 69, wherein the jacket comprises a hydrogel or thermoplastic material.

72. A pharmaceutical composition comprising

i) the polypeptide of any of the claims 1 to 21; or
ii) the isolated nucleic acid sequence of any of the claims 22 to 49; or
iii) the expression vector of any of the claims 50 to 54; or
iv) a composition of host cells according to any of the claims 55 to 61; or
v) a packaging cell line according to any of the claims 62 to 63; or
vi) an implantable biocompatible cell device according to any of the claims 64 to 71; and
vii) a pharmaceutically acceptable carrier.

73. The use of

i) the polypeptide of any of the claims 1 to 21; or
ii) the isolated nucleic acid sequence of any of the claims 22 to 49; or
iii) the expression vector of any of the claims 50 to 54; or
iv) a composition of host cells according to any of the claims 55 to 61;
v) an implantable biocompatible cell device according to any of the claims 64 to 71; or
vi) a packaging cell line according to any of the claims 62 to 63;

for the manufacture of a medicament.

74. The use of claim 73, wherein said medicament is for the treatment of an immunological disorder.

75. The use of claim 74, wherein the immunological disorder is selected from the group consisting of: infectious diseases, immune deficiencies, cancer, autoimmune disorders

including multiple sclerosis, allergic reactions and conditions, and graft-versus-host disease.

76. The use of claim 73, wherein said medicament is for the treatment of a disease, disorder, or damage associated with the nervous system.

77. The use of claim 76, wherein said medicament is for the treatment of a disease, disorder, or damage involving injury to the brain, brain stem, the spinal cord, and/or peripheral nerves, including but not limited to conditions such as stroke, traumatic brain injury, spinal cord injury, diffuse axonal injury, epilepsy, neuropathy, peripheral neuropathy and associated pain and other symptoms.

78. The use of claim 76, wherein the Nervous System disorder involves degeneration of neurons and their processes in the brain, brain stem, the spinal cord, and/or the peripheral nerves, including but not limited to Parkinson's Disease, Alzheimer's Disease, senile dementia, Huntington's Disease, amyotrophic lateral sclerosis, neuronal injury associated with multiple sclerosis, and associated symptoms.

79. The use of claim 78, wherein the neurodegenerative disease is Parkinson' Disease.

80. The use of claim 78, wherein the neurodegenerative disease is Huntington's Disease.

81. The use of claim 78, wherein the neurodegenerative disease is amyotrophic lateral sclerosis.

82. The use of claim 76, wherein the nervous system disorder is a disease, disorder, or damage involving dysfunction and/or loss of neurons in the brain, brain stem, the spinal cord, and/or peripheral nerves, including but not limited to conditions caused by metabolic diseases, nutiritional deficiency, toxic injury, malignancy, and/or genetic or idiopathic conditions including but not limited to diabetes, renal dysfunction, alcoholism, chemotherapy, chemical agents, drug abuse, vitamin deficiency, and infection.

83. The use of claim 82 or 77, wherein the disease is peripheral neuropathy and associated pain.

84. The use of claim 76, wherein the nervous system disorder is a disease, disorder, or damage involving degeneration or sclerosis of glia such as oligodendrocytes, astrocytes and Schwann cells in the brain, brain stem, the spinal cord, and the peripheral nerves, including but not limited to multiple sclerosis, optic neuritis, cerebral sclerosis, post-infectious encephalomyelitis, and epilepsy and associated symptoms.

85. The use of claim 84, wherein the disease or disorder is multiple sclerosis, sensory ataxus, neurodegenerative spinocerebellar disorders, hereditary ataxis, cerebellar atrophies, and alcoholism.

86. The use of claim 76, wherein the nervous system disorder, disease, or damage involves the retina, photoreceptors, and associated nerves including but not limited to retinitis pigmentosa, macular degeneration, glaucoma, diabetic retinopathy, and associated symptoms.

87. The use of claim 76, wherein the nervous system disorder, disease, or damage involves the sensory epithelium ans associated ganglia of the vestibuloacoustic complex including but not limited to noise-induced hearing loss, deafness, tinnitus, otitis, labyrintitis, hereditary and cochleovestibular atrophies, Menieres Disease, and associated symptoms.

88. A method of treatment of a pathological condition in a subject comprising administering to an individual in need thereof a therapeutically effective amount of:

i) the polypeptide of any of the claims 1 to 21; or

ii) the isolated nucleic acid sequence of any of the claims 22 to 49; or

iii) the expression vector of any of the claims 50 to 54; or

iv) a composition of host cells according to any of the claims 55 to 61; or

v) an implantable biocompatible cell device according to any of the claims 64 71; or

vi) a packaging cell line according to any of the claims 62 to 63.

89. The method of claim 88, wherein the pathological condition is an immunological disorder.

90. The method of claim 89, wherein the immunological disorder is selected from the group consisting of: infectious diseases, immune deficiencies, cancer, autoimmune disorders

including multiple sclerosis, allergic reactions and conditions, and graft-versus-host disease.

91. The method of claim 88, wherein said medicament is for the treatment of a disease, disorder, or damage associated with the nervous system.

92. The method of claim 91, wherein said medicament is for the treatment of a disease, disorder, or damage involving injury to the brain, brain stem, the spinal cord, and/or peripheral nerves, including but not limited to conditions such as stroke, traumatic brain injury, spinal cord injury, diffuse axonal injury, epilepsy, neuropathy, peripheral neuropathy and associated pain and other symptoms.

93. The method of claim 91, wherein the Nervous System disorder involves degeneration of neurons and their processes in the brain, brain stem, the spinal cord, and/or the peripheral nerves, including but not limited to Parkinson's Disease, Alzheimer's Disease, senile dementia, Huntington's Disease, amyotrophic lateral sclerosis, neuronal injury associated with multiple sclerosis, and associated symptoms.

94. The method of claim 93, wherein the neurodegenerative disease is Parkinson' Disease.

95. The method of claim 93, wherein the neurodegenerative disease is Huntington's Disease.

96. The method of claim 93, wherein the neurodegenerative disease is amyotrophic lateral sclerosis.

97. The method of claim 91, wherein the nervous system disorder is a disease, disorder, or damage involving dysfunction and/or loss of neurons in the brain, brain stem, the spinal cord, and/or peripheral nerves, including but not limited to conditions caused by metabolic diseases, nutiritional deficiency, toxic injury, malignancy, and/or genetic or idiopathic conditions including but not limited to diabetes, renal dysfunction, alcoholism, chemotherapy, chemical agents, drug abuse, vitamin deficiency, and infection.

98. The method of claim 97, wherein the disease is peripheral neuropathy and associated pain.

99. The method of claim 91, wherein the nervous system disorder is a disease, disorder, or damage involving degeneration or sclerosis of glia such as oligodendrocytes, astrocytes and Schwann cells in the brain, brain stem, the spinal cord, and the peripheral nerves, including but not limited to multiple sclerosis, optic neuritis, cerebral sclerosis, post-infectious encephalomyelitis, and epilepsy and associated symptoms.

100. The method of claim 99, wherein the disease or disorder is multiple sclerosis, sensory ataxus, neurodegenerative spinocerebellar disorders, hereditary ataxis, cerebellar atrophies, and alcoholism.

101. The method of claim 91, wherein the nervous system disorder, disease, or damage involves the retina, photoreceptors, and associated nerves including but not limited to retinitis pigmentosa, macular degeneration, glaucoma, diabetic retinopathy, and associated symptoms.

102. The method of claim 91, wherein the nervous system disorder, disease, or damage involves the sensory epithelium ans associated ganglia of the vestibuloacoustic complex including but not limited to noise-induced hearing loss, deafness, tinnitus, otitis, labyrintitis, hereditary and cochleovestibular atrophies, Menieres Disease, and associated symptoms.

103. The method of claim 88, wherein the subject is a human being.

104. A method of expanding a composition of mammalian cells, comprising administering to said composition the polypeptide of any of the claims 1 to 21; or transducing/transfecting the cells with the expression vector of any of the claims 50 to 54.

105. A method of differentiating a composition of mammalian cells, comprising administering to said composition the polypeptide of any of the claims 1 to 21; or transducing/transfecting the cells with the expression vector of any of the claims 50 to 54.

106. An antibody capable of binding to a polypeptide of any of the claims 1 to 21.

107. The antibody of claim 106, being selected from the group consisting of: polyclonal antibodies, monoklonal antibodies, humanised antibodies, single chain antibodies, recombinant antibodies.

108. An immunoconjugate comprising the antibody of claim 106 and a conjugate selected from the group consisting of: a cytotoxic agent such as a chemotherapeutic agent, a toxin, or a radioactive isotope; a member of a specific binding pair, such as avidin or streptavidin or an antigen; an enzyme capable of producing a detectable product.

109. An isolated polypeptide selected from the group consisting of AA_{128}-AA_{293} of SEQ ID No 3, AA_{121}-AA_{293} of SEQ ID No 3, AA_{129}-AA_{294} of SEQ ID No 8, AA_{122}-AA_{294} of SEQ ID No 8, AA_{126}-AA_{291} of SEQ ID No 13, AA_{119}-AA_{291} of SEQ ID No 13, and variant of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 15 of the amino acid residues in the sequence are so changed.

110. The isolated polypeptide of claim 109, wherein the changed amino acids are selected from those designated as unconserved, weakly conserved or strongly conserved in Figure 3.

111. An isolated polypeptide selected from the group consisting of SEQ ID No 19, 20, 21, 22, 23, and 24, and variant of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 15 of the amino acid residues in the sequence are so changed.

112. The isolated polypeptide of claim 111, wherein the changed amino acids are selected from those designated as unconserved, weakly conserved or strongly conserved in Figure 3.

113. An isolated polypeptide selected from the group consisting of:

i) AA_{30}-AA_{288} of SEQ ID No 3, and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{25}-AA_{293} of SEQ ID No 3;

ii) AA_{28}-AA_{286} of SEQ ID No 13 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{23}-AA_{291} of SEQ ID No 13;

iii) AA_{31}-AA_{289} of SEQ ID No 8 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{26}-AA_{294} of SEQ ID No 8; and

iv) variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 20 of the amino acid residues in the sequence are so changed.

114. An isolated polypeptide selected from the group consisting of:

i) AA_{171}-AA_{288} of SEQ ID No 3, and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{165}-AA_{288} of SEQ ID No 3;

ii) AA_{169}-AA_{286} of SEQ ID No 13 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{164}-AA_{291} of SEQ ID No 13;

iii) AA_{172}-AA_{289} of SEQ ID No 8 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, i.e. up to AA_{167}-AA_{294} of SEQ ID No 8;

iv) variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed.

115. An isolated polypeptide selected from the group consisting of:

i) AA_{30}-AA_{118} of SEQ ID No 3, and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{25}-AA_{123} of SEQ ID No 3;

ii) AA_{28}-AA_{116} of SEQ ID No 13 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{23}-AA_{121} of SEQ ID No 13;

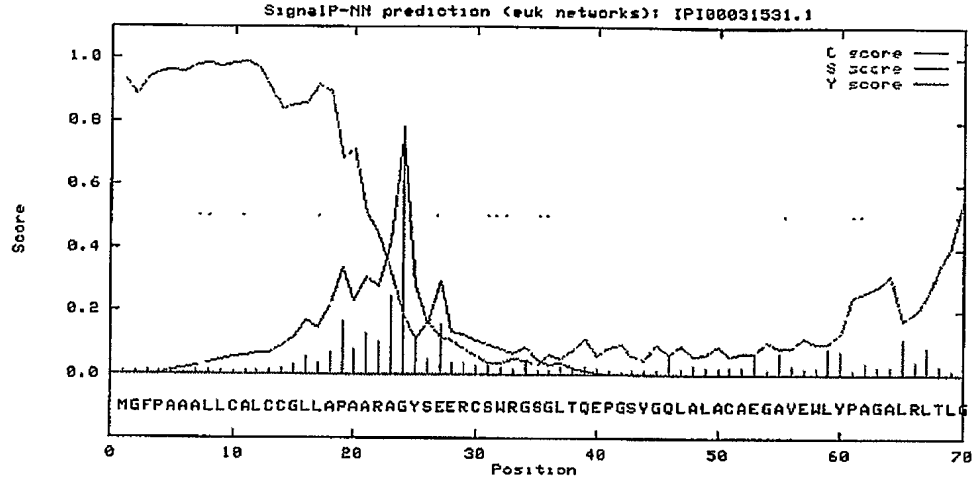
iii) AA_{31}-AA_{119} of SEQ ID No 8 and polypeptides having from one to five extra amino acids from the native sequence in one or both ends, up to AA_{26}-AA_{124} of SEQ ID No 8; and

iv) variants of said polypeptides, wherein any amino acid specified in the chosen sequence is changed to a different amino acid, provided that no more than 10 of the amino acid residues in the sequence are so changed.

116. The polypeptide of claim 113, 114, or 115, wherein the changed amino acids are selected from those designated as unconserved, weakly conserved or strongly conserved in Figure 3.

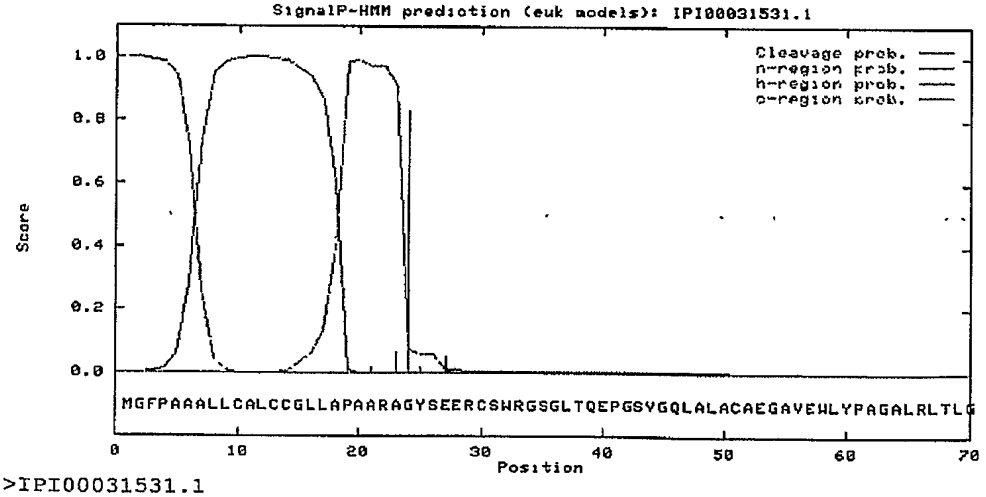
117. An isolated polynucleotide coding for a polypeptide according to any of claims 109 to 115.

Fig. 1A




```
>IPI00031531.1          length = 293
# Measure   Position  Value  Cutoff  signal peptide?
  max. C     24       0.785   0.33   YES
  max. Y     24       0.746   0.32   YES
  max. S     11       0.989   0.82   YES
  mean S      1-23    0.846   0.47   YES
# Most likely cleavage site between pos. 23 and 24: ARA-GY
```

Fig. 1B




```
>IPI00031531.1
Prediction: Signal peptide
Signal peptide probability: 1.000
Signal anchor probability: 0.000
Max cleavage site probability: 0.832 between pos. 23 and 24
```

FIG. 2

human NsG33 (SEQ ID No 3)

# Gene Ontology category	Odds
Signal_transducer	0.538
Receptor	0.433
Hormone	**1.173**
Structural_protein	0.168
Transporter	0.230
Ion_channel	0.372
Voltage-gated_ion_channel	0.130
Cation_channel	0.215
Transcription	0.294
Transcription_regulation	0.152
Stress_response	0.340
Immune_response	0.186
Growth_factor	**2.083**
Metal_ion_transport	0.020

human N-terminal peptide (SEQ ID No 19)

# Gene Ontology category	Odds
Signal_transducer	0.464
Receptor	0.296
Hormone	0.206
Structural_protein	0.987
Transporter	0.311
Ion_channel	0.147
Voltage-gated_ion_channel	0.157
Cation_channel	0.215
Transcription	0.311
Transcription_regulation	0.829
Stress_response	0.162
Immune_response	1.460
Growth_factor	**8.142**
Metal_ion_transport	0.061

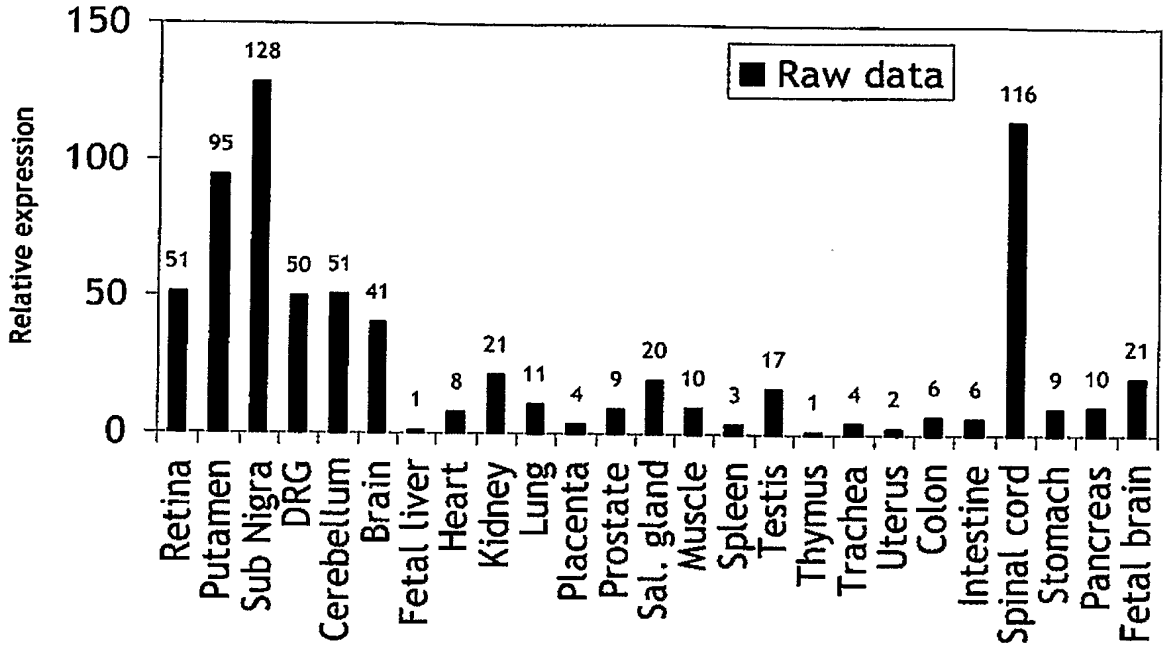
human C-terminal peptide (SEQ ID No 5)

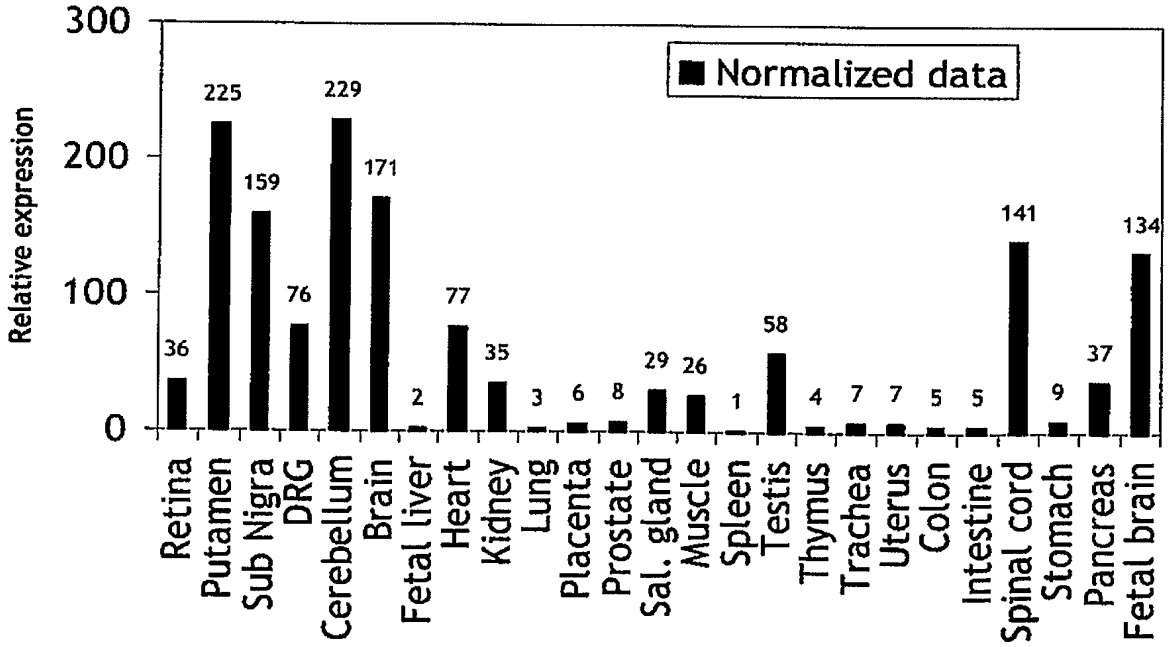
# Gene Ontology category	Odds
Signal_transducer	0.242
Receptor	0.038
Hormone	0.303
Structural_protein	0.096
Transporter	0.231
Ion_channel	0.185
Voltage-gated_ion_channel	0.191
Cation_channel	0.215
Transcription	0.312
Transcription_regulation	0.295
Stress_response	0.145
Immune_response	0.157
Growth_factor	**7.963**
Metal_ion_transport	0.020

Fig 3. CLUSTAL W (1.82) multiple sequence alignment

```
Mouse NsG33     HASAHASALLCALCCGLLAASAHAGYSEDRCSWRGSGLTQEPGSVGQLTLDCTEGAIEWL
Rat NsG33       ---MLVAALLCALCCGLLAASARAGYSEDRCSWRGSGLTQEPGSVGQLTLDCTEGAIEWL
Human NsG33     -MGPPAAALLCALCCGLLAPAARAGYSEERCSWRGSGLTQEPGSVGQLALACAEGAVEWL
                     .:************.:*:*****:**********************:* *:***:***

Mouse NsG33     YPAGALRLTLGGPDPGTRPSIVCLRPERPFAGAQVFAERMTGNLELLLAEGPDLAGGRCM
Rat NsG33       YPAGALRLTLGGSDPGTRPSIVCLRPTRPFAGAQVFAERMAGNLELLLAEGQGLAGGRCM
Human NsG33     YPAGALRLTLGGPDPRARPGIACLRPVRPFAGAQVFAERAGGALELLLAEGPGPAGGRCV
                ************.** :**.*.**** ************  * ******** . *****:

Mouse NsG33     RWGPRERRALFLQATPHRDISRRVAAFRFELHEDQRAEMSPQAQGLGVDGACRPCSDAEL
Rat NsG33       RWGPRERRALFLQATPHRDISRRVAAFQFELHEDQRAEMSPQAQGFGVDGACRPCSDAEL
Human NsG33     RWGPRERRALFLQATPHQDISRRVAAFRFELREDGRPELPPQAHGLGVDGACRPCSDAEL
                ******************:*********:***:** *.*:.***:*:***************

Mouse NsG33     LLAACTSDFVIHGTIHGVAHDTELQESVITVVVARVIRQTLPLFKEGSSEGQGRASIRTL
Rat NsG33       LLTACTSDFVIHGTIHGVVHDMELQESVITVVATRVIRQTLPLFQEGSSEGRGQASVRTL
Human NsG33     LLAACTSDFVIHGIIHGVTHDVELQESVITVVAARVLRQTPPLFQAGRSGDQGLTSIRTP
                **:********** ****.** **********.:**:*** ***: * * .:* :*:**

Mouse NsG33     LRCGVRPGPGSFLFMGWSRFGEAWLGCAPRFQEFSRVYSAALTTHLNPCEMALD
Rat NsG33       LRCGVRPGPGSFLFMGWSRFGEAWLGCAPRFQEFSRVYSAALAAHLNPCEVALD
Human NsG33     LRCGVHPGPGTFLFMGWSRFGEARLGCAPRFQEFRRAYEAARAAHLHPCEVALH
                *****:****:************ ********** *.*.** ::**:***:**.
```

Fig. 4





```
scoring matrix: BLOSUM50, gap penalties: -12/-2
42.3% identity;        Global alignment score: 747

                10        20        30        40        50 *
Innog. MRGAARAAWGRAGQPWPRPPAPGPPPPPLPLLLLLLAGLLGGAG-AQYSSDRCSWKGSGL
                                    :   ::  :  :::. :. : :: .::::.::::
NsG33  ------------------------MGFPAAALLCALCCGLLAPAARAGYSEERCSWRGSGL
                                      10        20        30

       60        70    *   80        90        100       *110
Innog. THEAHRKEVEQVYLRCAAGAVEWMYPTGALIVNLR-PNTFSPARHLTVCIRSFTDSSGAN
       :.:      : :. : :: :::::.::.::: ..:  :.    ::   .:.:     .:::.
NsG33  TQEPGS--VGQLALACAEGAVEWLYPAGALRLTLGGPDPR--ARPGIACLRPVRPFAGAQ
        40          50        60        70          80        90

      120        130       140  *       150       160       170
Innog. IYLEKTG-ELRLLVPDGDGRPGRVQC--FG-LEQGGLFVEATPQQDIGRRTTGFQYELVR
       .. :..:  :.::. .: : :.  .:  .:  :. .::..:::.:::.::...:..:: .
NsG33  VFAERAGGALELLLAEGPG-PAGGRCVRWGPRERRALFLQATPHQDISRRVAAFRFELRE
           100       110       120       130       140       150

             180         190       200       210       220
Innog. RHRAS---DLHELSAP--CRPCSDTEVLLAVCTSDFAVRGSIQQVTHEPERQDSAIHLRV
         :     . : :..   ::::::.:.::::.::::...: :. :::. : :.:.: . .
NsG33  DGRPELPPQAHGLGVDGACRPCSDAELLLAACTSDFVIHGIIHGVTHDVELQESVITVVA
           160       170*  *   180   *   190       200       210

     230       240       250         260       270       280
Innog. SRLYRQKSRVFEPVPEGDGHWQG--RVRTLLECGVRPGHGDFLFTGHMHFGEARLGCAPR
       .:. ::   .:.    ::   ::   .:: :.::::.:: : ::: :  .:::::::::::
NsG33  ARVLRQTPPLFQAGRSGD---QGLTSIRTPLRCGVHPGPGTFLFMGWSRFGEARLGCAPR
           220       230         240 *     250       260     *

       290       300     * 310
Innog. FKDFQRMYRDAQERGLNPCEVGTD
       :..:.: :. :.   :.:::::.
NsG33  FQEFRRAYEAARAAHLHPCEVALH
     270       280       290
```

Fig 5

```
   1 gcttcgccgg ggccgggcgg ccggcgcccc cggctgctcc cgccgccgcc cggacccgcg ccccgccggg gcagcggtgg tgagagcccc gactccccgg

 101 acgccgcccg ccgtgccatg gggttcccgg ccgcggcgct gctctgcgcg ctgtgctgcg gcctcctggc cccggctgcc cgcgccggct actccgagga
                    >>.....................................CDS.....................................>
                      m  g  f  p  a  a  a  l  l  c  a  l  c  c  g  l  l  a  p  a  a  r  a  g  y  s  e
 201 gcgctgcagc tggaggggca gcggcctcac ccaggagccc ggcagcgtgg ggcagctggc cctggcctgt gcggagggcg cggttgagtg gctgtacccg
     >.....................................CDS.....................................>
     e  r  c  s  w  r  g  s  g  l  t  q  e  p  g  s  v  g  q  l  a  l  a  c  a  e  g  a  v  e  w  l  y  p
 301 gctggggcgc tgcgcctgac cctgggcggc cccgatccca gagcgcggcc cggcatcgcc tgtctgcggc cggtgcggcc cttcgcgggc gcccaggtct
     >.....................................CDS.....................................>
       a  g  a  l  r  l  t  l  g  g  p  d  p  r  a  r  p  g  i  a  c  l  r  p  v  r  p  f  a  g  a  q  v
 401 tcgcggagcg cgcaggggcc gccctggagc tgctgctggc cgagggcccg ggcccggcag ggggccgctg cgtgcgctgg ggtccccgcg agcgccgggc
     >.....................................CDS.....................................>
      f  a  e  r  a  g  g  a  l  e  l  l  l  a  e  g  p  g  p  a  g  g  r  c  v  r  w  g  p  r  e  r  r
 501 cctcttcctg caggccacgc cgcaccagga catcagccgc cgcgtggccg ccttccgctt tgagctgcgc gaggacgggc gccccgagct gccccgcag
     >.....................................CDS.....................................>
     a  l  f  l  q  a  t  p  h  q  d  i  s  r  r  v  a  a  f  r  f  e  l  r  e  d  g  r  p  e  l  p  p  q
 601 gcccacggtc tcggcgtaga cggtgcctgc aggccctgca gcgacgctga gctgctcctg gccgcatgca ccagcgactt cgtaattcac gggatcatcc
     >.....................................CDS.....................................>
       a  h  g  l  g  v  d  g  a  c  r  p  c  s  d  a  e  l  l  l  a  a  c  t  s  d  f  v  i  h  g  i  i
 701 atggggtcac ccatgacgtg gagctgcagg agtctgtcat cactgtggtg gccgcccgtg tcctccgcca gacaccgccg ctgttccagg cggggcgatc
     >.....................................CDS.....................................>
      h  g  v  t  h  d  v  e  l  q  e  s  v  i  t  v  v  a  a  r  v  l  r  q  t  p  p  l  f  q  a  g  r
 801 cggggaccag gggctgacct ccattcgtac cccactgcgc tgtggcgtcc acccgggccc aggcaccttc ctcttcatgg gctggagccg ctttggggag
     >.....................................CDS.....................................>
     s  g  d  q  g  l  t  s  i  r  t  p  l  r  c  g  v  h  p  g  p  g  t  f  l  f  m  g  w  s  r  f  g  e
 901 gcccggctgg gctgtgcccc acgattccag gagttccgcc gtgcctacga ggctgcccgt gctgcccacc tccacccctg cgaggtggcg ctgcactgag
     >.....................................CDS.....................................>>
       a  r  l  g  c  a  p  r  f  q  e  f  r  r  a  y  e  a  a  r  a  a  h  l  h  p  c  e  v  a  l  h  -
1001 gggctgggtg ctggggaggg gctggtagga gggagggtgg gcccactgct ttggaggtga tgggactatc aataagaact ctgttcacgc aaaaaaaaaa

1101 aaaaaaaaa
```

Fig 6

```
   1  ccacgcgtcc gcccacgcgt ccgcgcttct ttgcgcgctc tgttgcggcc tcctggccgc gtccgctcac gctggctact cggaagaccg ctgcagctgg
       >>.....................................................CDS.....................................................>
         h  a  s  a  h  a  s  a  l  l  c  a  l  c  c  g  l  l  a  a  s  a  h  a  g  y  s  e  d  r  c  s  w
 101  aggggcagcg gtttgaccca ggagcctggc agcgtggggc agctgaccct ggactgtact gagggcgcta tcgagtggct gtacccagct ggggcgctgc
       >.....................................................CDS.....................................................>
         r  g  s  g  l  t  q  e  p  g  s  v  g  q  l  t  l  d  c  t  e  g  a  i  e  w  l  y  p  a  g  a  l
 201  gcctgaccct gggcggcccc gatccgggca cacggcccag catcgtctgt ctgcgcccag agcggccctt cgctggtgcc caggtcttcg ctgaacgtat
       >.....................................................CDS.....................................................>
         r  l  t  l  g  g  p  d  p  g  t  r  p  s  i  v  c  l  r  p  e  r  p  f  a  g  a  q  v  f  a  e  r
 301  gaccggcaat ctagagttgc tactggccga gggcccggac ctggctgggg gccgctgcat gcgctggggt ccccgcgagc gccgagccct ttcctgcag
       >.....................................................CDS.....................................................>
         m  t  g  n  l  e  l  l  l  a  e  g  p  d  l  a  g  g  r  c  m  r  w  g  p  r  e  r  r  a  l  f  l  q
 401  gccacaccac accgcgacat cagccgcaga gttgctgcct tccgttttga actgcacgag gaccaacgtg cagaaatgtc tccccaggct caaggtcttg
       >.....................................................CDS.....................................................>
         a  t  p  h  r  d  i  s  r  r  v  a  a  f  r  f  e  l  h  e  d  q  r  a  e  m  s  p  q  a  q  g  l
 501  gtgtggatgg tgcctgcagg ccctgcagtg atgccgagct cctcctggct gcatgcacca gtgattttgt gatccacggg accatccatg gggtcgccca
       >.....................................................CDS.....................................................>
         g  v  d  g  a  c  r  p  c  s  d  a  e  l  l  l  a  a  c  t  s  d  f  v  i  h  g  t  i  h  g  v  a
 601  tgacacagag ctgcaagaat cagtcatcac tgtggtggtt gctcgtgtca tccgccagac actgccactg ttcaaggaag ggagctcgga gggccaaggc
       >.....................................................CDS.....................................................>
         h  d  t  e  l  q  e  s  v  i  t  v  v  v  a  r  v  i  r  q  t  l  p  l  f  k  e  g  s  s  e  g  q  g
 701  cgggcctcca ttcgtacctt gctgcgctgt ggtgtgcgtc ctggcccagg ctccttcctc ttcatgggct ggagccgatt tggcgaagct tggctgggct
       >.....................................................CDS.....................................................>
         r  a  s  i  r  t  l  l  r  c  g  v  r  p  g  p  g  s  f  l  f  m  g  w  s  r  f  g  e  a  w  l  g
 801  gtgctccccg cttccaagag ttcagccgtg tctattcagc tgctctcacg acccatctca acccatgtga gatggcactg gactgagaga cctgggagca
       >.....................................................CDS.....................................................>>
         c  a  p  r  f  q  e  f  s  r  v  y  s  a  a  l  t  t  h  l  n  p  c  e  m  a  l  d  -
 901  agccctggat ggaccttctt ctggagatgg ggtgttgggg agggtgatgg gagggtgggt gagaagggtg tggctcggat ggcatcctgg tacccacagt

1001  gagctggtag aatactaagt aatctggacc ataaaaaaaa aaaaaaaa
```

Fig 7

```
   1  atgctggtag cggcgcttct ctgcgcgctg tgctgcggcc tcttggctgc gtccgctcga gctggctact ccgaggaccg ctgcagctgg aggggcagcg
      >>.....................................................CDS.....................................................>
        m  l  v  a  a  l  l  c  a  l  c  c  g  l  l  a  a  s  a  r  a  g  y  s  e  d  r  c  s  w  r  g  s
 101  gtttgaccca ggaacctggc agcgtggggc agctgaccct ggattgtact gagggtgcta tcgagtggct gtatccagct ggggcgctgc gcctgactct
      >.....................................................CDS.....................................................>
        g  l  t  q  c  p  g  s  v  g  q  j  t  l  d  c  t  c  g  a  i  c  w  l  y  p  a  g  a  l  r  l  t
 201  aggcggctct gatccgggca cgcggcccag catcgtctgt ctgcgcccaa cacggccctt cgctggtgcc caggtcttcg ctgaacggat ggccggcaac
      >.....................................................CDS.....................................................>
        l  g  g  s  d  p  g  t  r  p  s  i  v  c  l  r  p  t  r  p  f  a  g  a  q  v  f  a  e  r  r  a  g  n
 301  ctagagttgc tactggccga gggccaaggc ctggctgggg gccgctgcat gcgctggggt cctcgcgagc gccgagccct tttcctgcag gccacgccac
      >.....................................................CDS.....................................................>
        l  c  l  l  _  a  c  g  q  g  l  a  g  g  r  c  m  r  w  q  p  r  e  r  r  a  l  f  l  q  a  t  p
 401  accgggacat cagccgcaga gttgctgcct tccaatttga actgcacgag gaccaacgtg cagaaatgtc tccccaggcc caaggttttg gtgtggatgg
      >.....................................................CDS.....................................................>
        h  r  d  i  s  r  r  v  a  a  f  q  f  e  l  h  e  d  q  r  a  e  m  s  p  q  a  q  g  f  g  v  d
 501  tgcctgcagg ccctgcagtg atgccgagct ccttctgact gcatgcacca gtgactttgt gatccatggg accatccatg gggtcgtcca tgacatggag
      >.....................................................CDS.....................................................>
        q  a  c  r  p  c  s  d  a  e  l  l  l  t  a  c  t  s  d  f  v  i  h  g  t  i  h  g  v  v  h  d  m  e
 601  ctgcaagaat cagtcatcac tgtggtggcc actcgtgtca tccgccagac actgccactg ttccaggaag ggagctcgga gggccggggc caggcctccg
      >.....................................................CDS.....................................................>
        l  q  e  s  v  i  t  v  v  a  t  r  v  i  r  q  t  l  p  l  f  q  e  g  s  s  e  g  r  g  q  a  s
 701  ttcgtacctt gttgcgctgt ggtgtgcgtc ctggcccagg ctccttcctc ttcatgggct ggagccgatt tggcgaagct tggctgggct gcgctccccg
      >.....................................................CDS.....................................................>
        v  r  t  l  l  r  c  g  v  r  p  g  p  g  s  f  l  f  m  g  w  s  r  f  g  e  a  w  l  g  c  a  p
 801  cttccaagag ttcagccgtg tctattcagc tgctctcgcg gcccacctca acccatgtga ggtggcactg gactgagaga cctgggagca agccctggat
      >.....................................CDS.....................................>>
        r  f  q  e  f  s  r  v  y  s  a  a  l  a  a  h  l  n  p  c  e  v  a  l  d  -
 901  ggatcttcct ctggggatgg ggtgttgggg aggggtgata ggagggtggg tgggaagggt gtggctcaga tggcatcctg gtacccacag tgaggtggta

1001  gaatactaaa taacctggat cacacc
```

Fig 8